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Environmental surveillance of Legionellosis within an Italian University Hospital. Results of 15 years of analysis.

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Abstract: Legionnaires' disease is normally acquired by inhalation of legionellae from a contaminated environmental source. Water systems of large buildings, such as hospitals, are often contaminated with legionellae and therefore represent a potential risk for the hospital population. In this study, we demonstrated the constant presence of *Legionella* in water samples from the water system of a large university hospital in Messina (Sicily, Italy) consisting of 11 separate pavilions during a period of 15 years (2004-2018). In total, 1346 hot water samples were collected between January 2004 and December 2018. During this period, to recover *Legionella* spp. from water samples the standard procedures reported by the Italian Guidelines emanated in 2000 were adopted; from May 2015 to 2018 Italian Guidelines revised in 2015 (ISS, 2015), were used. The most water samples (72%) were positive to *L. pneumophila* serogroups 2-14 whereas *L. pneumophila* serogroup 1 accounted for the 18% and *Legionella* spp. for the 15%. Most of the positive samples were found in the buildings where are situated critical wards as ICU, Neurosurgery, Surgeries, Pneumology and Neonatal Intensive Unit Care. We highlighted the importance of a continuous monitoring of hospital water samples to prevent the potential risk of nosocomial legionellosis.

Keywords: Legionella; legionellosis; environmental surveillance; water system.

1. Introduction

The *Legionella* genus currently includes 61 saprophytic species and about 70 serogroups (sgrs), of which at least 20 have been recognized as responsible for opportunistic infections in human beings. The most frequently isolated species from cases of infection is *L. pneumophila*, which is subdivided into 16 different sgrs, of which the serogroup (sgr) 1 is the most pathogenic and causes 70% to 90% of all cases of legionellosis [1-3]. In contrast, *L. pneumophila* sgrs 2-14 account for only 15 to 20% of community-acquired cases, although they account for over 50% of the isolates obtained from man-made aquatic systems [4]. In Italy, since 1990 Legionellosis has been included among infectious diseases with compulsory notification (Class II: major diseases due to high frequency and/or subject to control actions) [5]. When a new case of legionellosis is diagnosed, physicians notify to the local health authority that provides to inform the Regional and consequently National Authorities.

The number of cases of legionellosis has increased steadily over the years, both in Europe and in Italy, in the latter due mainly to the numerous receptive structures present in the territory [6]. In 2015, in Italy, most

cases were community acquired (78,8%), followed by travel-associated (12,7%) and health care-associated (5,3%) cases. Particularly, in Sicily, in 2015, were diagnosed 19 new cases of legionellosis of which 18 were community-acquired and 1 was a travel-associated case; no one nosocomial case was notified [7].

In hospital and other health care facilities, waterborne diseases may originate from the bacterial colonization of water pipes, taps, cooling towers, showers and water supplies [8-14]. Moreover, previous studies investigated the role of unusual sources of *Legionella* in hospital, such as bubblers for oxygen-therapy, pediatric incubators, dental chairs, etc. [15] and in communities environment [16-18]. For hospitalized subjects, risk assessment based on levels of exposure to contaminated water pipes should be calculated following constant environmental monitoring, and critically with strict clinical surveillance [19,20]. *Legionella* is able to survive for long periods in water and even to replicate in the presence of chlorine, if it manages to create suitable conditions (areas of stagnation and sludge formation, parasitism of amoebas and protozoic cysts, etc.) [7,21]. Water systems represent suitable environments for the growth and multiplication of *Legionella* spp. and other Gram-negative bacteria, which survive at different pHs and temperatures [22-26].

The risk of illness increases dramatically if the germ is found in certain wards such as Intensive Care Unit, Hematology-Oncology Unit, Cardiology, Haemodialysis, Pulmonology for critical conditions of their hospitalized patients [27-32].

The interest of our laboratory to *Legionella* was born in 1988. At first, it was involved only in research activities aimed at recovery of *Legionella* in the small hospitals, care homes and other types of structures in Messina. Since 2004, it began to carry out a real surveillance at the University Hospital "G. Martino" and in 2102, was appointed the Regional Reference Laboratory of Clinical and Environmental Surveillance of Legionellosis, branch of Messina (Italy), situated within the Polyclinic area.

The purpose of this paper is to present a retrospective study of pluri-annual surveillance conducted by our Laboratory. Particularly, we demonstrated the constant presence of *Legionella* in water samples during a considered period and, consequently, we highlighted the importance of a continuous monitoring of hospital water samples to prevent the potential risk of nosocomial legionellosis. We present the results of surveillance conducted for 15 years, from 2004 to 2018. Moreover, we carried out, during the same period, the clinical surveillance of legionellosis through the direct research of the urinary antigen in clinical specimens.

2. Materials and Methods

As mentioned earlier, environmental monitoring of *Legionella* in the water pipelines within the University Hospital in Messina began in 1988. After a few years of desultory analysis, we went to a real continuous environmental surveillance with constant monitoring of all the pavilions that make up the structure. Monitoring is also active now.

2.1. Sampling.

From January 2004 to December 2018, 1346 samples were collected from the water distribution system of the "G. Martino" University Hospital (Messina, Italy) and were examined for *Legionella*. Monthly samplings were performed in 11 different buildings hosting the various wards. The groundwater is provided by the municipality and disinfected with chlorine dioxide; the water reaches the hospital by means of a single pipeline that leads to a centralized tank where the water is stored. The water does not undergo further chlorination after it is gathered from the Messina town pipeline. From the centralized tank, the water is distributed to each building by electric-motor pumps that send it through a pipeline that runs across the basements of all the buildings. Under each building there is a boiler which produces heated water (average temperature approximately 13–48° C) that climbs up again to supply the wards located on each floor. Samples of heated water were collected at the start of daily activities from taps using 1 L-sterile glass bottles. In order

to obtain a sampling representative of the hygienic-sanitary conditions, care was taken to sample all the floors, and the wards located both on the left and on the right sides inside each building. Particularly, we collected the water samples from taps of bathrooms located inside and outside the wardrooms (i.g. aisles, waiting rooms), ambulatories, laboratories, operating, sterilization, storage and medical/nursing rooms.

2.2. Isolation and serological identification of *Legionella* spp.

From January 2004 to April 2015, to recover *Legionella* spp. from water samples the standard procedures reported by the Italian Guidelines emanated in 2000 [33] were adopted; from May 2015 to 2018 Italian Guidelines revised in 2015 were used [3]. In the last years, in according to the guidelines, 1 L of water samples were concentrated to 10 mL through 0.2 μ m porosity membrane filters and incubated at 50°C for 30 min in a thermostatic bath. Concentrated and unconcentrated samples were spread on duplicate plates of Buffered Charcoal-Yeast Extract Agar Base Medium (BCYE, Oxoid Ltd., Milan, Italy) and incubated for 10 days at 36–37 °C in a moist chamber with 2.5% CO₂. The suspected colonies were isolated and confirmed as *Legionella* spp. after screening their inability to grow on a culture medium without cysteine. *Legionella* counts were reported in Colony Forming Units/liter (CFUs/L) according to the number of colonies per plate and to the dilutions performed on the original sample. A latex micro-agglutination Test Kit with polyvalent antisera (Oxoid) was used to identify the isolates assumed to belong to *Legionella* genus. For serological identification also “*Legionella pneumophila* monovalent antisera set 1 and 2” and *Legionella* antisera for several *Legionella* species as *L. bozemani*, *dumoffii*, *gormanii*, *micdadei*, etc (Biogenetics, Tokio, Japan) were used.

3. Results

Figure 1 shows the percentages of samples collected during the entire period in each building of the studied University Hospital.

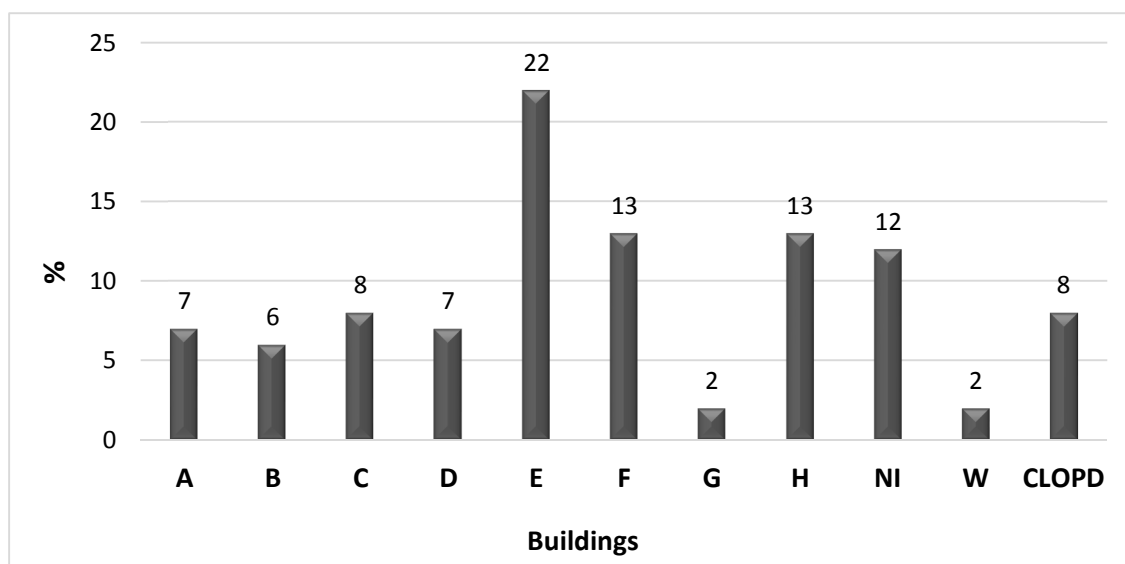


Figure 1. Percentages of samples collected in each building.

The figure shows that the most part of the water samples were collected in the buildings where are situated the “critical” wards as ICU, Neurosurgery, Surgeries (general and specialized), Pulmunology and Neonatal Intensive Unit Care. Particularly, the building where we collected the most part of samples was the building

E (21%) for the presence of the ICU and Neurosurgery, followed by the buildings H (Pulmonology, Infectious Diseases, Hematology, Oncology), F (General Surgery) and NI (Pediatrics), with percentages of 13%, 12% and 12% respectively.

Of 1346 collected water samples, 812 (60%) were positive. Table 1 shows the different building, the principal medical activities that take place in their context, the total positivity and the different serotypes isolated in each building. Of all samples, 42 (3%) were positive to more serotypes at the same time. Particularly, 9 (21%) were positive to *L. pneumophila* 1 + *L. pneumophila* 2-14; 14 (34%) were positive to *L. pneumophila* 1 + *Legionella* spp; 16 (38%) were positive to *L. pneumophila* 2-14 + *Legionella* spp and only 3 (7%) were positive to all the tested strains.

Building	Medical activities	Total samples	Negative samples	Positive samples	Samples positive for LP 1	Samples positive for LP 2-14	Samples positive for <i>Legionella</i> spp
A	Obstetrics and Gynecology	87 (6%)	25 (29%)	62 (71%)	4 (6%)	44 (71%)	17 (27%)
B	Internal Medicine	67 (5%)	29 (43%)	38 (57%)	0	21 (55%)	20 (53%)
C	Cardiology-Nephrology	95 (7%)	68 (72%)	27 (28%)	0	26 (96%)	1 (4%)
D	Pathological Anatomy	76 (6%)	37 (49%)	39 (51%)	2 (5%)	37 (95%)	4 (10%)
E	ICU-Neurology-Neurosurgery-Orthopedics	280 (21%)	64 (23%)	216 (77%)	112 (52%)	101 (47%)	28 (25%)
F	General Surgeries	163 (12%)	69 (42%)	94 (58%)	2 (2%)	88 (94%)	5 (5%)
G	Laboratories	136 (10%)	52 (38%)	84 (62%)	26 (31%)	58 (69%)	2 (2%)
H	Pulmonology, Infectious Diseases, Thoracic and Vascular Surgeries, Oncology	173 (13%)	41 (24%)	132 (76%)	0	118 (89%)	14 (11%)
NI	Pediatrics	160 (12%)	60 (38%)	100 (62%)	1 (1%)	93 (93%)	10 (10%)
W	Ambulatories	13 (1%)	11 (85%)	2 (15%)	0	1 (50%)	1 (50%)
CLOPD	Dentistry	96 (7%)	78 (81%)	18 (19%)	0	1 (5%)	17 (95%)

TOTAL	1346	534	812	147	588	119
(%)		(40%)	(60%)	(18%)	(72%)	(15%)

Table 1. Different building, principal medical activities, total positivity and different serotypes isolated by each building.

The most water samples (72%) were positive to *L. pneumophila* serotypes 2-14 whereas *L. pneumophila* serotype 1 accounted for the 18%. A certain percentage of samples (15%) was positive to *Legionella* spp. The total percentage is not equal to 100% because we found different *Legionella* serotypes in the same sample. The buildings where we found a major positivity rate were the E (77%), H (76%) and A (71%), followed by distance from the others. The most sampled sites were taps of bathrooms situated outside the wardrooms (29%) and ambulatories (28%), followed by taps of wardrooms (17%) and laboratories (14%). Concerning the bacterial load, we divided the isolates in four groups:

- 1 = ≤ 1000 CFU/L;
- 2 = 1001-10.000 CFU/L;
- 3 = 10.001-100.000 CFU/L;
- 4 = ≥ 100.000 CFU/LI).

The most part of *L. pneumophila* 1 isolated (82%) was included in the first two groups; the rest (18%) in the third. *L. pneumophila* 2-14 isolates had a load belonging to the second group for the 37% while the first and the third group were in similar percentages equal to 29% and 31% respectively; a little amount of isolates (3%) belonging to the fourth group. Finally, *Legionella* spp isolates belonging especially to the first group (44%) with the second and the third group similarly represented (27% and 24% respectively. Only 5% of isolates belonging to the fourth group (Figure 2).

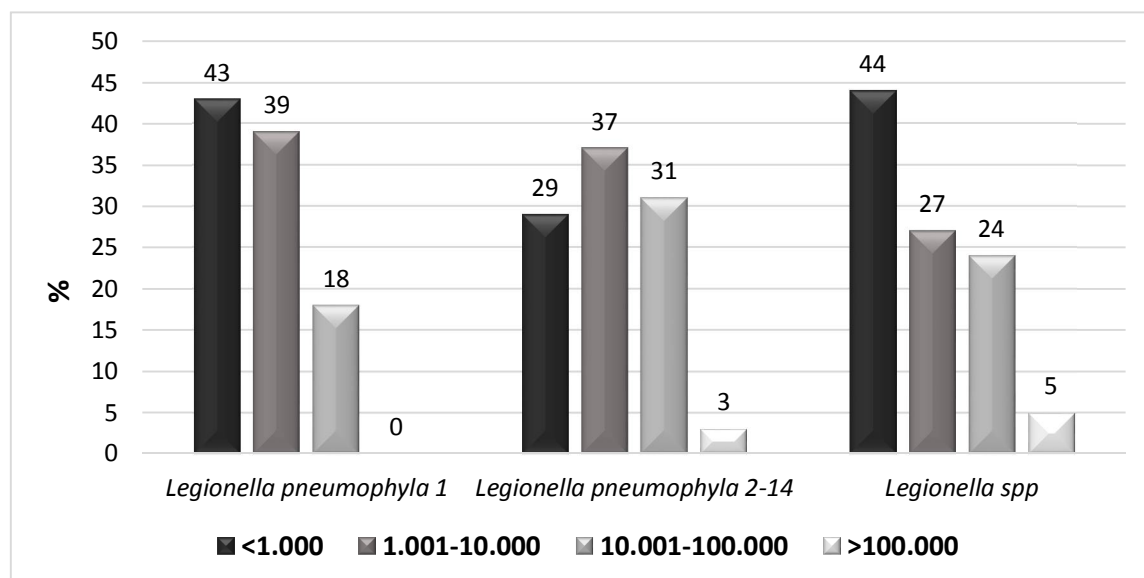


Figure 2. Bacterial load of the different *Legionella* serotypes isolated by water samples.

Figure 3 shows the positivity trend during the considered period.

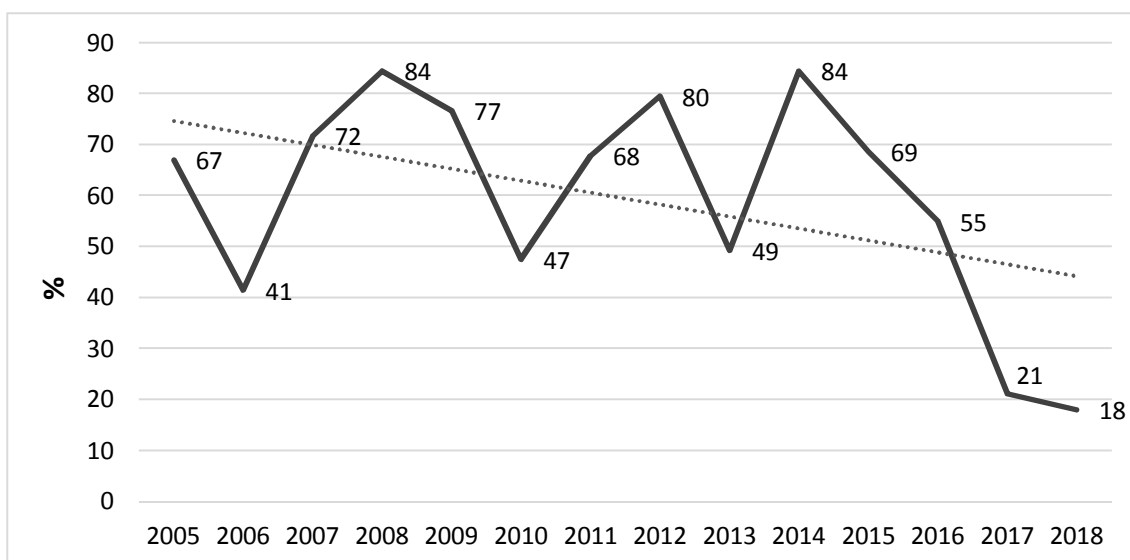


Figure 3. Positivity trend of Legionella isolates in the considered period

The figure shows a rather constant presence of positive samples during the considered years but an evident decreasing trend in the last years. Figure 4 shows the trend of the different isolated *Legionella* serotypes.

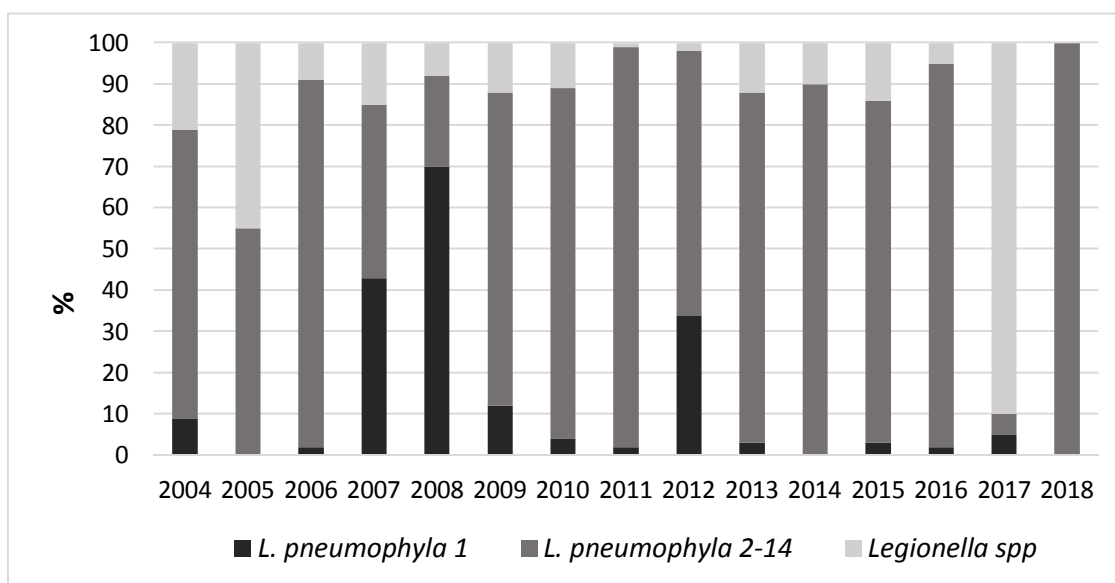


Figure 4. Positivity of different isolated Legionella serotypes.

4. Discussion

Legionellae are microorganisms that have their natural habitat in the water and can easily reach the pipelines, where they can reproduce favored by the presence of biofilm in the inner walls of the pipes. In the network of hot water distribution, often the temperature coincides with the optimum for the replication of

these bacteria. Further favorable factors are the length and the tortuosity of the hot water distribution network normally present in the big buildings like hospitals.

The Messina University Hospital is a large structure that extends over an area of about 310,000 m² and it is distributed on 11 pavilions, indicated by letters of the alphabet (Figure 5), which have four to six floors raised and which house the various environments both university and hospital services Department (as ambulatories, hospital rooms, operating rooms, laboratories, changing rooms for doctors and nurses, kitchens and services, classrooms, libraries, refectory, etc).



Figure 5. University Hospital of Messina map.

The construction of the first pavilion dates back to 1967, but it is an ever-evolving structure with expansions and modifications dictated by the requirements of adapting to the most modern welfare standards. However, the open building sites promote the presence and the growth of *Legionella*. Moreover, the renovation often does not involve the water pipelines that remain those of origin. For all these reasons, it is not surprising that levels of significant contamination have been found in most of the health structure where similar surveys were conducted [34-36]. The presence of fouling in the pipes and dead points with stagnation of the water in the networks well explain the limited effectiveness of water chlorination and thermal treatments. Indeed, the

eradication of *Legionellae* contamination in hospital is very difficult due to the common failure of the decontamination interventions of the water network [37,38]. One of the possible solution to prevent Legionella spread from the contaminated water is surely the application of tap filters that allows having water free of bacterial contamination for all uses even if it involves a certain cost.

In this study, our surveillance demonstrates the constant presence of Legionella strains in the water supplied in a big hospital structure during a long period of time (15 years). The surveillance was carried out especially in the buildings where are located critical wards as ICU, Neurosurgery and Oncology. The more important issue to highlight is that these buildings were those where we found the most positivity rate. In these wards, the inpatients are often immune-compromised and they are, consequently, at high risk to contract many infections. This result shows that these patients have a potential high risk to contract a legionellosis with subsequent worsening of the already compromised health conditions. This issue is true especially concerning inpatients of ICU and Neurosurgery wards, hospitalized in the building E, where we found a large presence of *L. pneumophila* 1 (52%), known to be the most pathogen strain of the genus. However, as shown in Figure 2, for this strain we found a bacterial load lower to 10.000 CFU/ml.

To prevent nosocomial cases of legionellosis, national and international guidelines recommend measures to control *Legionella* water contamination, with particular reference to health care structures [34,39]. In particular, for *Legionella* control it is important focused the attention on three basic parameters: (1) the amount of the germ in the water, through analytical monitoring; (2) the presence of virulence factors in isolated strains; (3) the receptivity in the guests [40]. As the World Health Organization (WHO) suggests, the best approach to assessing the health risks associated with exposure to *Legionella* is the development of a Water Safety Plan (WSP) that is one operational tool useful for systematic assessment of pipelines or risk analysis. Particularly, the plan is a dynamic tool that gives priority to the risks and dangers of pipelines colonization and considers the most appropriate control measures as well as the possible obstacles to their realization.

Despite the environmental surveillance was constant and thorough during all these 14 years, the parallel clinical surveillance was, on the contrary, poor and limited to few urine samples. The wards that have proved to be more careful to this issue were ICU, Pneumology, Infectious Diseases and Internal Medicine. However, despite this attention, the adherence to this survey is still far away to be satisfactory. We can deduce that there is still a long way to go. This issue is index of a remarkable under-notify of Legionella infections still present even among the healthcare workers. Probably, physicians are unused to think to *Legionella* as possible cause of nosocomial pneumonias. It is necessary a continuous education of the healthcare workers in order to improve the situation, to understand the real incidence of legionellosis and, especially, to obtain a correct diagnosis and treatment of this infection.

5. Conclusions

This paper emphasizes the need to develop a risk analysis aimed at controlling and preventing *Legionella* in all community structures. In particular, through the description of the surveillance actions carried out, the Regional Reference Laboratory for Legionellosis of Messina suggests to identify specific Critical Control Points (CCPs) according to the logic of HACCP. Moreover, due to the evidence of *Legionella* presence even in the aerosol, in very recent years we have associate the control of this latter to the routine water control (data not yet published). Indeed, many recent studies suggest and recommend the concurrent research water/air to increase the probability of finding the microorganism [41-43]. This aspects are important not only in order to plan the management and monitoring actions of the pipelines, but also in view of a Public Health approach, with the specific aim of preventing illnesses in the exposed population.

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