

# ASSESSMENT OF THE PRESENCE OF *Legionella* spp. IN THE AQUATIC ENVIRONMENTS OF MARRAKESH MOROCCO

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## **Abstract**

Legionellosis is a collection of infections that emerged in the 20th century, and that are caused by *Legionella* bacteria. Water is the major natural reservoir for bacteria belonging to *Legionella* genus, and are found worldwide. About 20% of cases of legionellosis detected in Europe are considered to be travel-related. However, no studies have assessed the incidence of legionellosis in Morocco.

The aim of our work is the isolation of *Legionella* strains from different natural and artificial Moroccan aquatic environments in order to assess the risk and understand the relationship of these bacteria with their ecosystems.

Thirty samples were collected from fifteen aquatic sites of Marrakech regions (dams, wells, steam, waste water, fountains, lakes and basin). The physicochemical and microbiological characterizations of these samples were carrying out. *Legionella* were isolated and purified using the GVPC medium.

We have isolated strains of *Legionella* from three of the fifteen inspected aquatic sites. From 30 tested water samples coming from these sites, *Legionella* was isolated in 7 water samples, 5 of which had been collected from wells and 2 from a basin.

From a microbiological point of view, the contaminated sites are characterized by the absence of fecal coliforms and fecal streptococci, and by the presence of *Aeromonas* and *Pseudomonas*. For physicochemical properties, they are characterized by a medium rate of oxygen, a temperature ranging from 26 to 35 °C and a pH ranging from 7 to 8.

This study demonstrates presence of *Legionella* in Marrakesh water and the importance of assessing water quality.

**Keywords:** *Legionella*, isolation, Marrakesh, Aquatic environment.

## Introduction

Many water borne outbreaks are related to anthropogenic water Systems and are mainly due to protozoan parasites and bacterial species like *Legionella* spp., *Pseudomonas* spp. and *Aeromonas* spp., all commonly found in biofilms (Szewzyk et al., 2000).

*Legionella pneumophila*, which is known as the causative agent of a severe and possibly fatal

pneumonia called Legionnaires'disease, has already caused the death of thousands of individuals world-wide since its association with human illness in 1976 (Fields et al., 2002).

*Legionella* spp. are ubiquitous. They are found in natural aquatic environments (streams, rivers, ponds, lakes and thermal pools) in moist soil and in mud. The organisms are able to survive in moist environments for long periods of time and can withstand temperatures of 0 and 68°C and a pH range of 5.0 and 8.5. They can survive chlorination and thus enter water supply systems and proliferate in thermal habitats, including air-conditioning cooling towers, hot water systems, shower heads, taps, whirlpool spas and respiratory ventilators. The objective of this study is the isolation of *Legionella* strains from different natural and artificial Moroccan aquatic environments in order to assess the risk and understand the relationship of these bacteria with their ecosystems.

## Methods

Thirty samples were collected from fifteen aquatic sites of Marrakech regions (two dams, six wells, two streams, one wastewater plant, two fountains, one lake and one basin).

### Isolation of *Legionella* strains

Water samples were analyzed using a modified International Standard method, ISO/DIS 11731, 1996 (Water Quality—Detection and Enumeration of *Legionella*). One liter of each sample was concentrated aseptically by membrane filtration, using cellulose membranes with a pore size of 0.45 µm. The concentrated samples were removed by cutting the membranes into four pieces, placing them in sterile containers containing 3 mL of the original sample and sonicating them in an ultrasound tank (Ultrasons-H) for 10 min until the membrane as appeared to be clean.

The concentrated samples were divided in three aliquots of one milliliter each: the first-one; untreated, the second-one; heat-treated (50 °C for 30 min), and the last-one; acid-treated (in 9 mL of HCl-KCl acid buffer at pH 2.2 for 5 min). Of each aliquot, 0.1 mL was plated onto GVPC (glycine, vancomycin, polymyxin B, cyclohexamide) selective agar medium (Biolife). Plates were incubated at 36 °C for 10 days and examined for growth every 48 h. Colonies morphologically consistent with *Legionella* spp. were plated onto buffered charcoal yeast extract (BCYE) agar (Biolife) and BCYE agar without cystein medium (Biolife). The colonies, which grew on BCYE agar with cystein but not on BCYE agar without cystein, were considered as *Legionella* spp. (Lasheras et al., 2006).

### Physical and chemical analysis

Standard techniques were used to measure all physical and chemical parameters (pH, temperature, Dissolved oxygen, conductivity) described in Standard Methods for the Examination of Water and Wastewater (APHA, 2000).

## Bacteriological quality control of water samples

Samples for determination of water bacteriological quality were simultaneously collected, maintained at 4 °C and examined within 6h. Water samples were filtered through Millipore filters (0.45 mm pore size).

Total coliforms and fecal coliforms were enumerated on Lactose TTC agar with tergitol 7 medium (Biokar), fecal streptococci were enumerated on BEA agar (Biokar), *Aeromonas* spp were enumerated on PADE medium (Imzilin et al., 1997) and *Pseudomonas aeruginosa* were enumerated on Cetrimide agar (Biokar) and confirmed as described in Standard Methods for the Examination of Water and Wastewater (APHA, 2000)..

## Results and discussion

A total of 15 sites were examined, and 30 samples were collected. Tree sites (20%) and Seven (23.33%) of samples were *Legionella*-positive (Table 1). Certain water characteristics such as high oxygen rates and the presence of *Aeromonas* were associated with the presence of *Legionella* species but did not promote their proliferation. The necessity of oxygen to the survival of *Legionella* was largely reported (Zahar and Kouatchet, 2008). Conversely, temperature and pH were not correlated with the presence of *Legionella* species but did promote their proliferation. It is reported that the temperature of the water is a factor related to the multiplication and a major determinant of *Legionella* colonization (Zanetti et al., 2000). Water quality had an important role on the presence and proliferation of *Legionella* species (Table.1). The contaminated sites are characterized by the absence of fecal coliforms and fecal streptococci, and the presence of *Pseudomonas* and especially *Aeromonas* which can be explained by the fact that these species form biofilms (Szewzyk et al., 2000).

Table 1. Association between presence of *Legionella* species and physicochemical and microbiological characteristics of water

Sites	Total Coliforms	Fecal Coliforms	fecal streptococci	<i>Aeromonas</i>	<i>Pseudomonas</i>	pH	Température °C	Conductivité (µs)	Oxygène dissous (mg/l)	<i>Legionella</i>
Well 1	+	-	-	+++	+	7,4	26,1	1573	13,32	+
Well 2	+	-	-	++	-	7,6	17	1398	13,14	+
Well 3	+	-	-	+	+	7,1	26	3230	6,75	-
Well 4	+	-	+	+	+	7,24	29,9	2760	6,02	-
Well 5	+	-	-	+	+	7,45	32	624	4,95	-
Well 6	-	-	-	+	-	8	25	392	10,7	-
Lakes 1	+	-	-	+	-	8,46	29,8	7456	5,98	-
Lakes 2	-	-	-	-	+	8,69	36	355	8,06	-

Dams 1	-	-	-	+	-	8,57	16	333	7,54	-
Dams 2	+	+	+	+	-	8,67	34	364	7,93	-
waste water	+++	++	++	+++	-	7,51	35	1332	8,6	-
Fountain 1	+	-	-	+	-	8,55	17,6	407	13,9	-
Fountain 2	+	-	-	+	-	8,3	20	640	12,6	-
Steam 1	-	-	-	-	+	7,56	35,4	158	10,33	-
Steam 2	+	-	-	+	+	7,44	28	641	10,42	-
Basin	+	-	-	++	-	8,31	35	623	12,85	+

## Conclusion

As a whole, this study demonstrates presence of *Legionella* in Marrakesh water and the importance of assessing water quality when evaluating environmental risk factors and in selecting the most appropriate prevention and control measures public water systems. This is important because contamination within these settings has been clearly linked to outbreaks of Legionnaires' disease.

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