



Assessment of the presence of *Legionella* spp. in some aquatic environments of Marrakesh Morocco

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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. *Legionella* spp. are ubiquitous. They are found in natural aquatic environments (streams, rivers, ponds, lakes and thermal pools) in moist soil and in mud.

Methods

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

Physical and chemical analysis:

Standard techniques were used to measure all physical and chemical parameters (pH, temperature, Dissolved oxygen, conductivity).

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, fecal streptococci were enumerated on BEA agar, *Aeromonas* spp were enumerated on PADE medium (Imziin et al., 1997) and *Pseudomonas aeruginosa* were enumerated on Cetrimide agar.

Isolation of *Legionella* strains:

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. 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 and BCYE agar without cystein medium. 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).

Results and discussion

| Sites | <i>Legionella</i> |
|-------------|-------------------|
| Well 1 | + |
| Well 2 | + |
| Well 3 | - |
| Well 4 | - |
| Well 5 | - |
| Well 6 | - |
| Lakes 1 | - |
| Lakes 2 | - |
| Dams 1 | - |
| Dams 2 | - |
| waste water | - |
| Fountain 1 | - |
| Fountain 2 | - |
| Steam 1 | - |
| Steam 2 | - |
| Basin | + |

Table 1 : Sites containing *Legionella*

A total of 15 sites were examined, and 30 samples were collected. Three sites (20%) and Seven (23.33%) of samples were *Legionella*-positive (Table 1).

Certain water characteristics such as high oxygen rates and were associated with the presence of *Legionella* species but did not promote their proliferation (Table 2). 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).

| Sites | pH | Temperature °C | Conductivity (µs) | Dissolved Oxygen (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 | + |

Table 2 : Association between presence of *Legionella* species and physicochemical characteristics of water.

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 (Table 3) (Szewzyk et al., 2000).



Figure 1 : *Legionella* strains isolated on GVPC medium

| Sites | Total Coliforms | Fecal Coliforms | fecal streptococci | <i>Aeromonas</i> | <i>Pseudomonas</i> | <i>Legionella</i> |
|-------------|-----------------|-----------------|--------------------|------------------|--------------------|-------------------|
| Well 1 | + | - | - | +++ | + | + |
| Well 2 | + | - | - | ++ | - | + |
| Well 3 | + | - | - | + | + | - |
| Well 4 | + | - | + | + | + | - |
| Well 5 | + | - | - | + | + | - |
| Well 6 | - | - | - | + | - | - |
| Lakes 1 | + | - | - | + | - | - |
| Lakes 2 | - | - | - | - | + | - |
| Dams 1 | - | - | - | + | - | - |
| Dams 2 | ++ | + | + | ++ | - | - |
| waste water | +++ | ++ | ++ | +++ | - | - |
| Fountain 1 | + | - | - | + | - | - |
| Fountain 2 | + | - | - | + | - | - |
| Steam 1 | - | - | - | - | + | - |
| Steam 2 | + | - | - | + | + | - |
| Basin | + | - | - | ++ | - | + |

Table 3 : Association between presence of *Legionella* species and physicochemical characteristics of water.

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.

References

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