Identification of *Legionella* Bacteria in Hot Water Supply of West Bank's Hospitals

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Dedication

To my Parents
Who Inspired me to Understand the Deep Values of patience, Encouragement, and Success

To My Dear Brothers and Sisters, and
To My Lovely Husband

To all the People Supported, and Encouraged me

Acknowledgment

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Abstract

An evaluation was made of the prevalence of *Legionella* species in hot water distribution systems in West Bank hospitals and their possible association with *Pseudomonas aeruginosa*.

Legionella and P. *aeruginosa* was investigated in this study in six West Bank hospitals - Ramallah, Alia- Hebron, Beit Jalla, Alwatani, Rafidia, and Jenin – representative of different region of West Bank (Northern, Central, and Southern West Bank). A total of 134 water samples were collected (53 samples for *Legionella* analysis, and 81 samples for *P. aeruginosa*).

L. pneumophila sg (2-14) was isolated from 33 (62.3%) of 53 samples that were analyzed. In the positive samples, the mean number of *L. pneumophila* sg (2-14) was 6.17 x 10^3 CFU/L with range from 100 CFU/L to 2.85 x 10^4 CFU/L

P. aeruginosa were isolated from 17 (21%) of 81 samples, with levels ranging from 1 CFU/200 mL to TNTC CFU/200 mL.

To assess the effect of heat disinfection on *L. pneumophila* sg (2-14), samples were taken from hospital tap water systems before and after thermal disinfection. In Biet Jalla hospital, the water system was heated to 80°C and held at this temperature for 30 minutes, all distal outlets were flushed with this hot water, positive samples with *L.*

pneumophila were reduced from 100% (before heat disinfection) to 17% (after heat disinfection). At Jenin hospital thermal disinfection was conducted at 70°C for 30 minutes, the concentration of *Legionella* was reduced, but not killed completely.

It was demonstrated that the high number of *Legionella* in water distribution systems can be successfully reduced by heat treatment, but not totally killed. However, thermal disinfection at 70°C for 30 minutes in Jenin hospital was successfully efficient for the elimination of all *P. aeruginosa* in water distribution systems, there was a reduction in *P. aeruginosa* positive samples from 100% to 0%.

In this study it was noticed that all the pediatric divisions in West Bank govermental hospitals were contaminated with either *P. aeruginosa* or *L. pneumophila* sg (2-14) or both, which is considered a real health hazard to children's health. Also the faucet in incubators room where premature babies bath in are contaminated.

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List of Abbreviations

APHA: American Public Health Association

ATCC: American Type Culture Collection

°C: Degree Celsius

BCYE: Buffer Charcoal Yeast Extraction

CDC: Centers for Disease Control and Prevention

CF: Cystic Fibrosis

CFU: Colony Forming Unit

CPHL: Central Public Health Laboratory

DAF: Direct Fluorescent Antibody

DHS: Department OF Health Services

ICU: Intensive Care Unit

L: Liter

LAC: Los Angeles Country

L. pneumophila: Legionella pneumophila

L. pneumophila sg (2-14)*: Legionella pneumophila* serogroup (2-14)

L. pneumophila sg 1*: Legionella pneumophila* serogroup 1

L. P (2-14): *Legionella pneumophila* serogroup (2-14)

LD: Legionnaires' Disease

ml: milliliter

mm: millimeter

MOH: Ministry of Health

NWY: Modified Wadowsky Yee

P. aeruginosa: *Pseudomonas aeruginosa*

TNTC: Too Numerous To Count

µm: micro meter

WHO: World Health Organisation

Chapter One

Introduction

Background

Hospital Water Supply

Water is one of the most effective vehicles to transfer pathogens. The water system within the hospital is the most frequent source of cases or outbreaks where patients may be at a higher risk for a severe infection (Borella *et al* 2004).

Point-of-use water (faucets and showers) may be the source of the transmission of waterborne microorganisms. Patient may be exposed to waterborne pathogens while showering, bathing, drinking water, and from exposure to medical equipment rinsed with potentially contaminated tap water, or the hands of medical personnel washed with contaminated tap water (Pall Medical 2003), as shown in Fig.1.1

Sources of organisms include:

• Hospital water storage tanks

• Faucet tap water

• Showers (Pall Medical ,2003).

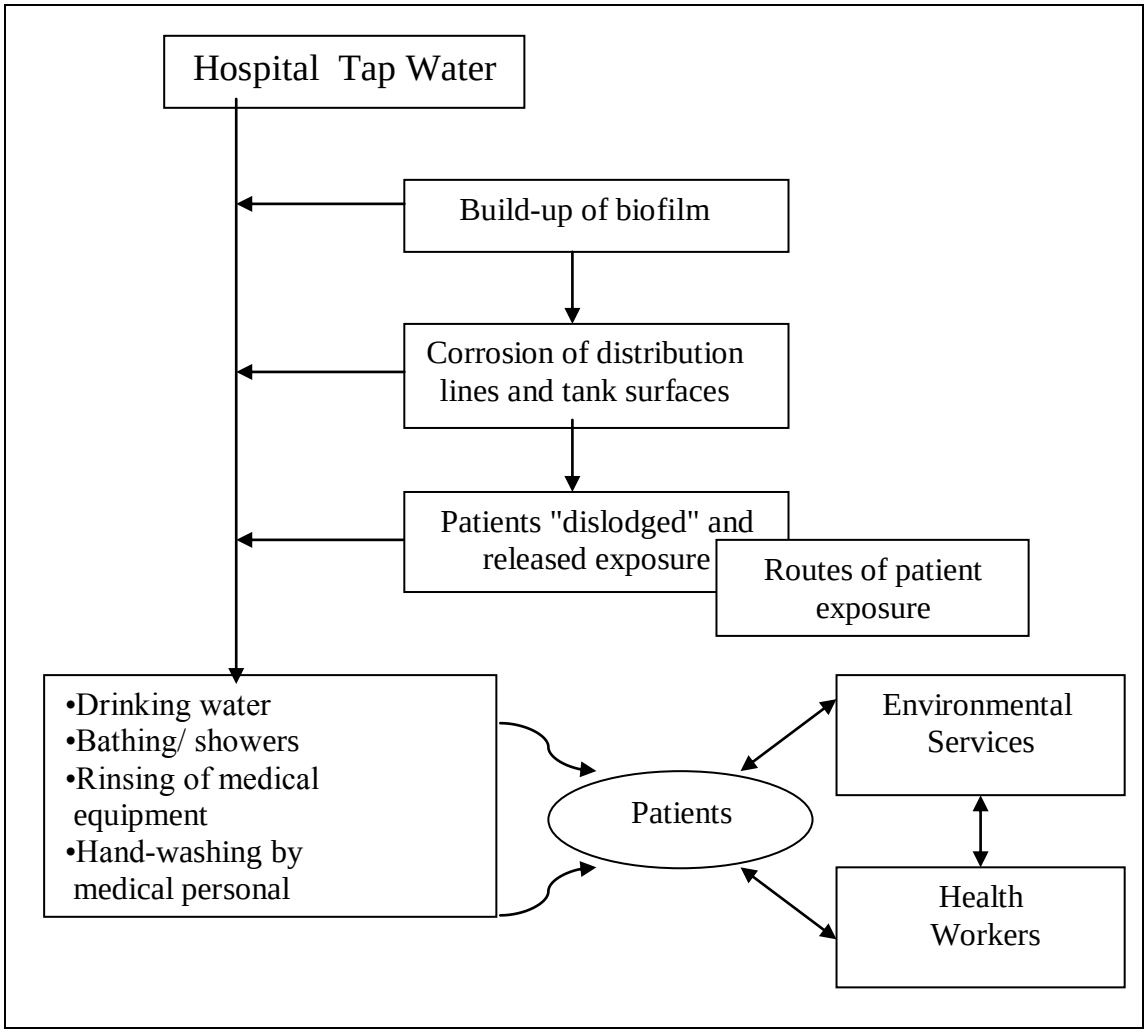

Fig.1.1: Point-of-use water in the hospital setting may be a source of the transmission of waterborne pathogens. source: Anaissie and others (2002)

The primary cause of poor water quality is the build-up of biofilm, corrosion of the distribution system and tank surfaces, aging systems, and water stagnation. Biofilm forms on any surface exposed to water and bacteria.

Problem

Contamination of the hospital water supply with potentially pathogenic organisms (*Legionella pneumophila* and P*seudomonas aeruginosa*) is very common world-wide, and is a well-known risk factor for nosocomial infection (Leoni *et al* 2005; Visca *et al* 1999). Large numbers of these pathogens in water distribution systems present a potentially serious health risk to workers and the patients, but the magnitude of the problem is largely unrecognized and there are no specific guidelines for protecting patients from exposure (Pall Medical 2003).

No study was done previously in Palestine about the determination of hospital water contamination with *L. pneumophila* and *P. aeruginosa* which are considered as opportunistic pathogens that cause nosocomial infection. The purpose of this study is to identify and quantify the levels of *Legionella* contamination in West Bank hospitals water supplies, Furthermore, recovery of *Legionella* from environmental water samples contain *Pseudomonas spp* sometimes is difficult because *Pseudomonas spp.* secrete into surrounding media bacterial products that can inhibit *Legionella* growth (American Public Health Association APHA 1998), thus *P. aeruginosa* colonization was evaluated.

Objectives

The objectives of this study can be summarized as follows:

1. To estimate the frequency of *Legionella* and *P. aeruginosa* colonization of water in West Bank hospitals, and severity of contamination.

2. To define the relationship between water distribution systems age and the contamination with *Legionella* and *P. aeruginosa*.

3. To assess the effect of thermal disinfection to reduce the contamination of *Legionella* and *P. aeruginosa*.

4. To provide policy makers with a clear idea about the hospital water contamination with *Legionella* and *P. aeruginosa* in order to take a suitable action to solve this problem.

5. To examine availability of biofilm in distribution water system by taking swabs from interior surface of faucets and showerhead and analyze it.

Chapter Two

Literature Review

Legionella bacteria

Water is the major natural reservoir for the *Legionella* organisms. The presence of *L .pneumophila* in hospital water supplies is a well-known risk factor for nosocomial pneumonia. Large numbers of *L. pneumophila* in water distribution systems present a potentially serious health risk to workers and the public (Bartie *et al* 2001; Leoni *et al* 2005).

No study has been conducted in West Bank regarding *Legionella* in water distribution systems in hospitals. Given the fact that hot water is supplied in hospitals, the possibility for *Legionella* growths in places like bathrooms and the distribution system and the consequent infections cannot be ignored. The present study is an attempt to determine the frequency of *Legionella* in the water consumed in general hospitals in the West Bank.

The genus *Legionella*, a member of the family legionellaceae, At least 42 species have been identified, 22 currently known species have been linked to human diseases. *L. pneumophila* is the most pathogenic of the species, causing up to 90% of the cases of legionellosis. *L. pneumophila* serogroup1 is most frequently associated with human disease, is thought to be responsible for 80% of the reported cases of legionellosis caused by *L pneumophila*. In addition, *L. pneumophila* is found to include at least 14

serotype, with serogroups 1, 4, and 6 being the primary causes of human disease. Other serogroups of *L. pneumophila* and occasionally other *Legionella* have also been reported to cause disease (American Public Health Association APHA 1998; World Health Organization WHO 1996; Yang 2004; Borella *et al* 2004; Sullivan L.E and Coleman D. 2004).

The *Legionella* bacterium was first identified in the summer of 1976 outbreak of febrile pneumonia occurred during the 58th annual convention of the American Legion, which was held at the Bellevue-Stratford Hotel in Philadelphia. Infection was presumed to be spread by contamination of the water in the hotel's air conditioning system (Ketchum 1988; Sullivan L.E. and Coleman D. 2004; Stout *et al* 1997). The presentation of affected persons ranged from mild flu like symptoms to multisystem organ failure. Of the 221 people infected, 34 died (Sullivan L.E. and Coleman D. 2004; O'Flanagan, *et al* 2002). Significant efforts were made at the Centers for Disease Control and Prevention (CDC) to identify the etiologic agent. A previously unknown bacterium was isolated and demonstrated in 1977 to be the etiologic agent responsible for causing the disease. It was named *Legionella* (to honor the victims of the Legionnaires' convention) *pneumophila* (pneumonia symptoms and occurred in Philadelphia) (Yang 2004; Forbes *et al* 2002).

Legionellosis is a recently described infectious disease of humans occurring world-wide, caused by exposure to *Legionella spp*. According to CDC an estimation of 8,000-18,000 cases occur each year in the United States, but only a fraction of these are reported (Attar *et al* 2004). The number of nosocomial infections due to *L .pneumophila* is underestimated in many countries.

Legionella are gram-negative, aerobic, rod-shaped, non-spore-forming bacteria, they are 0.5 to 0.7μm wide and 2 to 20 μm long. They posses polar, subpolar, and /or lateral flagella. All required L- cysteine and iron salts and buffered to pH 6.9 for optimum growth and primary isolation (Forbes *et al* 2002; American Public Health Association APHA 1998, World Health Organization WHO 1996).

Legionella bacteria are considered to have a worldwide distribution, although outbreaks of Legionnaires' disease are a more common occurrence in the northeast United States, England, Australia, the Netherlands, and a few other countries. Many regions of the US have had reports of Legionnaires' disease. Because of the wide distribution, close attentions should be paid to monitoring the presence and amplification of *Legionella* bacteria and to their control in building water systems. (Yang 2004)

Nature of *Legionella* Bacteria

Legionella are widespread in natural sources of water and may also be found in soils. On account of its tolerance to heat and low nutritional needs, it capable of surviving in extreme range of environmental conditions for long periods. *Legionella spp*. have been isolated from the majority of natural water sources investigated, including lakes, rivers, and marine waters, as well as moist soil. Organisms are also widely distributed in man-made water systems, particularly in hot-water and cooling-water systems. The microorganism passes from its natural reservoirs into the water distribution network, where the main source of contamination can be found; and therefore it poses a serious public health risk (Forbes *et al* 2002; World Health Organization WHO 1996; Borella *et al* 2004; Leoni *et al* 2005).

Legionella can survive in a temperature range of 20-50ºC, and multiply in the laboratory at temperatures between 20 and 46ºC., although there are indications that they can survive in temperatures lower than 20ºC, at 60ºC start to kill (World Health Organization WHO 1996) but in other study (Leoni *et al* 2005) it can remain viable at temperatures even up to 60ºC . Temperatures favorable for growth may be found in cooling-towers, spas, cold-water systems in buildings, hot-water system operated below 60ºC or "dead legs" of such systems operated at higher temperatures. It is also clear that high temperatures, over 60ºC can be used to control *Legionella* bacteria (World Health Organization WHO 1996; Leoni *et al* 2005).

The ecology of this group of bacteria is important in their spread. They are water-borne and can be aerosolized. It was found that *L. pneumophila* may survive as long as 139 days at room temperature in distilled water and for over a year in tap water. They grow, not just survive, in tap water in association with amoebae. The organisms can survive in aerosols and have been found as far as 200 m away from the aerosol source (Yang 2004).

Legionella species living inside of amoeba in hospital water. While amoeba typically feed on bacteria, some bacteria such as *Legionella* spp and *Pseudomonas* spp resist digestion by amoeba and live inside the amoeba. So the amoeba in the hospital water distribution system becomes a "Trojan horse" delivering the risk of nosocomial exposure and disease while resisting commonly used disinfectants such as chlorine. (Angelbeck 2004)

Routs of Exposure

Legionnaires' disease (LD) is normally acquired by inhalation or aspiration of *Legionella* from a contaminated environmental source. Water systems of large buildings, such as hotels, and hospitals, are often contaminated with *Legionella* and therefore represent a potential danger to patients. Several reports have shown a clear association between the presence of *Legionella* in hot water systems and the occurrence of legionellosis . The degree of *Legionella* contamination in hospital water

supplies has been shown to correlate with the incidence of nosocomial Legionnaires' disease (Wellinghausen *et al* 2001; Stout *et al* 1997).

Legionella infections are acquired exclusively from environmental sources; no person-to-person spread has been documented. Infection is the result of the inhalation of aerosols as shown in Fig. 2.1 that are small enough to penetrate the lungs (1 to 5µm diameter) and be retained by the alveoli. *Legionella* concentrations of 3–7,000 CFU/L could be sufficient to produce one case per year in a susceptible population, Exposure to these aerosols can occur via aspiration, respiratory therapy equipment, or wounds infected with contaminated water. In addition, transmission has been linked to the use of humidifiers, nebulizers filled with tap water and items that merely were rinsed with contaminated tap water (Pancer *et al* 2003; Forbes *et al* 2002; World Health Organization WHO 1996; Bollin *et al* 1985; Sullivan L.E. and Coleman D. 2004; Borella *et al* 2004).

The dose of Legionella necessary for infection is unknown, but the infective dose for susceptible humans can be assumed to be low, as patients have been known to be infected after exposure of only a few minutes to the sources of some outbreaks (World Health Organization WHO 2005).

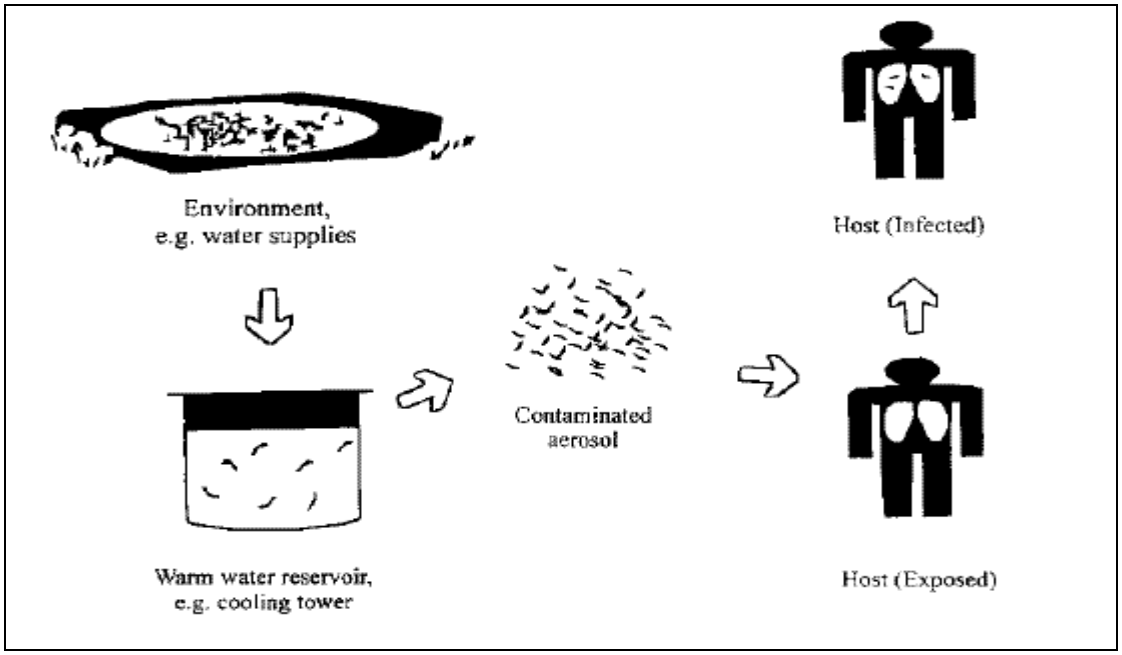

Fig.2.1 Chain of transmission of *Legionella* (source: Cameron *et al* 1996)

The degree of risk depends on four key factors

1. The density of the bacteria in the source;
2. The extent of aerosol generation;
3. The number of inhaled bacteria, depends on the size of the aerosol generated <5 μm being most dangerous, the dispersal of the aerosol in the air, and
4. The susceptibility of the exposed individual (World Health Organization WHO 1996).

Health Risk

Legionella bacteria cause two forms of disease: Legionnaires' disease (LD) and non-pneumonic Legionnaires' disease (Pontiac fever). They are collectively called legionellosis. Both diseases are transmitted through airway exposure, and there has been no report of human-to-human transmission. *Legionella* bacteria in airborne water droplets from contaminated water sources are the primary source of human exposure (American Public Health Association APHA 1998; World Health Organization WHO 1996; Yang 2004).

Legionnaires' disease is caused by several species of *Legionella* bacteria (about 50% of the cases of Legionnaires' disease). The disease is a form of pneumonia with an incubation period usually of 3-6 days and includes symptoms of pneumonia (World Health Organization WHO 1996). Legionnaires' disease is normally acquired by inhalation or aspiration of *Legionella* from a contaminated environmental source (Borella *et al* 2004). Risk factors include smoking, alcohol abuse, cancer, diabetes, chronic respiratory or kidney disease, older than 60, and sever immunosuppression, as in transplant recipients. Nosocomial Legionnaires disease also has been reported among patients in pediatric hospitals Usually less than 5% of exposed people develop the disease. On occasion, the fatality rate in untreated cases may be 10% -20% (World Health Organization WHO 1996; Yang 2004; Forbes *et al* 2002; Centers for Disease Control and Prevention CDC 1997).

Underlying disease and advanced age are risk factors not only for acquiring Legionnaires disease but also for dying as a result of the illness. In a multivariate analysis of 3,524 cases reported to CDC from 1980 through 1989, immunosuppression, advanced age, end-stage renal disease, cancer, and nosocomial acquisition of disease were each independently associated with a fatal outcome. The mortality rate was 40% among 803 persons who had nosocomially acquired cases, compared with 20% among 2,721 persons who had community-acquired cases; this difference probably reflected the increased severity of underlying disease in hospitalized patients (Centers for Disease Control and Prevention CDC 1997).

Legionnaires' disease can be treated effectively with antibiotics (e.g. erythromycin, rifampicin, azithromycin, levofloxacin, etc.) if it is diagnosed quickly. The diagnosis is based on pneumonia symptoms and confirmed with chest x-ray and various laboratory diagnostic tests for evidence of recent exposure to *Legionella* bacteria. Common laboratory tests include isolation and confirmation of *Legionella* bacteria from a clinical specimen; a four-fold increase of antibody titer against *Legionella* bacteria; detection of *Legionella* bacteria in a clinical sample using the direct fluorescent antibody assay (DFA); and the detection of *Legionella* specific antigen in urine (available only for limited *Legionella* species) (Yang 2004).

Legionnaires' disease is uncommon, but common-source outbreaks attract much attention. Between 100-200 cases are reported each year In England, and Wales, and in Germany; in France, the incidence is somewhat higher, with over 400 cases per year. Hospital-associated Legionaires' disease is the most serious form, because it

usually affects debilitated persons and has a high mortality rate (World Health Organization WHO 1996).

The non-pneumonic form of the disease is milder, with a higher attack rate, an acute onset (5 hours to 3 days) and symptoms similar to those of influenza: fever headache, nausea, vomiting, aching muscles, and coughing. No fatal cases have been reported and few outbreaks have been recognized. (Yang 2004; World Health Organization WHO 1996)

An estimated 8,000-18,000 legionellosis cases occur each year in the United States, but only a fraction of these are reported. Most LD cases are sporadic; 23% are nosocomial and 10%-20% can be linked to outbreaks. Pontiac fever has been recognized only during outbreaks. (Centers for Disease Control and Prevention CDC 2005)

Furthermore, *Pseudomonas* may compete with *Legionella* to grow in the aquatic environment; (Borella, *et al* 2004) thus we also evaluated *Pseudomonas* colonization. So recovery of *Legionella* from environmental water samples contain *Pseudomonas spp* sometimes is difficult because *Pseudomonas spp.* secrete into surrounding media bacterial products that can inhibit *Legionella* growth (American Public Health Association APHA 1998) therefore this study will investigate *P. aeruginosa* contamination also in hospital's water systems.

Control of *Legionella*

Most outbreaks of Legionnaires' disease are caused by *L. pneumophila* sg 1 and 6. Several CDC Guideline on Infection Control measures now call for no *Legionella* to be present in hospital areas (Emmerson 2001).

The objective of *Legionella* prevention is not elimination of *Legionella* in water systems, but ensuring the absence of conditions that foster bacteria amplification that may lead to disease transmission (Country of Los Angeles Department of Health Services Public Health 2003).

Therefore, routine and systematic monitoring can serve as an alarm system to determine whether there is contamination and if remediation is necessary or not.

The Centers of Disease Control and Prevention (CDC) outline two prevention strategies:

1. Routine periodic culturing of water samples from the hospital's potable water system for the purpose of detecting *Legionella* species, and

2. Maintain a high index of suspicion for LD among all nosocomial pneumonia cases, especially in individuals at high risk for this disease, and pursue an environmental investigation upon confirmation of one definite case or two

probable cases (Country of Los Angeles Department of Health Services Public Health 2003).

In the event the hospital water systems are contaminated, several remediation and control methods may be used. The following are the main remediation and control methods:

1- The super-chlorination method is to introduce free chlorine (Cl) gas into the water system and allow the increased Cl levels to circulate the entire water system for a few hours. All outlets are opened and flushed so proper disinfection can be achieved. Monitoring of free Cl from selected outlets is done to ensure that there is an effective free Cl level in the water. A free Cl concentration of greater than 5ppm is recommended for super-chlorination. In addition, a chlorine gas injector may be installed to constantly introduce Cl gas into the water system to maintain a free Cl level at 1-2 ppm to control any re-contamination. Chlorine gas, although it is highly effective at concentrations over 5-10 ppm, is corrosive to the plumbing system at the 2 ppm level or higher. In addition, Cl gas is not stable at high temperatures and may produce chlorinated organics, which are potentially carcinogenic (Yang 2004; Emmerson 2001).

2- The super-heating method is to raise the hot water temperature to at least 70ºC or higher. The hot water is to circulate and flush the entire water system and the outlets for a period of time. There is no standard duration for allowing the super-hot water to flush the system. Flushing for 5 to 30 minutes at 70ºC has been

suggested. However, this does not take into consideration the age of the plumbing system and the thickness of accumulated biofilm, which is not a good heat conductor. In addition to ensuring sufficient duration of flushing, the hot water temperature must be properly maintained (Yang 2004; Emmerson 2001).

3- Using filters that remove bacteria. The CDC recommends that the water filters have 0.2 μm filtration capability. Filters are successful in filtering bacteria and can be placed easily on faucets and showers, see Fig. 2.2.

Fig. 2.2: 0.2 μm water filters can be placed easily on faucets and showers (source: Pall Medical 2003)

Pseudomonas aeruginosa

Almost 50 years ago, *P. aeruginosa* was rarely considered as a real pathogen. In the 1970s it was recognized as the microorganism associated with bacteraemia in the neutropenic host. Nowadays, it is among the most common pathogens involved in nosocomial infections (Giamarellou 2002). The role of *P. aeruginosa* as an important pathogen in children, especially in premature infants, has been known since 1960 (Faco *et al* 2000).

P. aeruginosa are part of the natural population of the water, it a predilection for growth in moist environments (Borella *et al* 2004) *P. aeruginosa* is primarily a nosocomial pathogen.

In the United States 1,400 deaths occur each year in hospitals as a result of waterborne nosocomial pneumonias caused by *P. aeruginosa* alone which represented for 9–11% of all nosocomial infections with no clear guidelines for prevention of these infections (Anaissie *et al* 2003).

It is tolerant to a wide variety of physical conditions, including temperature, chlorination, and it is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics, which contribute to its ecological success and to its role as an effective opportunistic pathogen (Kenneth 2004).

P. aeruginosa is often found in wet hospital environments such as tap water, showers, sinks, soap, disinfectants, , medical equipment such as respiratory equipment, dialysis equipment, humidifiers, water in ventilators and incubators, water baths, and suction apparatuses and can multiply in these environments and then transfer to compromised patients (Matar *et al.* 2005). The hands of health workers have been considered as reservoir for *P. aeruginosa* infection among patients especially infants in a neonatal intensive care unit (Faco *et al* 2000).

Despite the advances in hospital care and the introduction of a wide variety of antimicrobial agents, *P. aeruginosa* continues to be a major nosocomial pathogen particularly in patients who suffer from immunodeficiency. *P. aeruginosa* is a ubiquitous pathogen prevalent in the hospital environments, and can cause severe nosocomial infections (Matar et al 2003). It causes urinary tract infections, respiratory system infections, pneumonia, dermatitis, soft tissue infections, sepsis (blood stream infection), and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. *P. aeruginosa* infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, *P. aeruginosa* is also one of the most common burn wound pathogens and has been shown to colonize or infect over one-fifth of burn patients. The case fatality rate in these patients is 50 percent. Pseudomonas colonizes the lungs of patients with cystic fibrosis (CF) and contributes to the chronic progressive pulmonary disease (Matar *et al.* 2003; Kenneth 2004; Barbeau *et al* 1998;).

P. aeruginosa was recognized as the predominant Gram-negative species (28.7%) isolated from bronchopulmonary infection sites of patients hospitalized in 1417 intensive care units (ICUs) of 17 Western European countries (Giamarellou 2002).

P. aeruginosa is ubiquitous in soil and water, and on surfaces in contact with soil or water. It is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. Almost all strains are motile by means of a single polar flagellum (Kenneth 2004).

P. aeruginosa has very simple nutritional requirements. It is often observed "growing in distilled water" which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of *P. aeruginosa* consists of acetate for carbon and ammonium sulfate for nitrogen (Kenneth 2004).

Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees.

Biofilms

The primary cause of poor water quality is the build-up of biofilm, corrosion of the distribution system and tank surfaces, aging systems, and water stagnation. Biofilm forms on any surface exposed to water and bacteria. They are communities of microorganisms adhering to environmental surfaces surrounded by the slime they secrete. *P. aeruginosa* and *Legionella* are from the "pioneers" in creating the biofilm seen in water distribution systems (Pall Medical 2003).

Aquatic biofilms, which are widespread not only in nature but also in medical and dental devices, can be the source of serious nosocomial infections.

Steps in Biofilm Development:

Step 1: Surface conditioning:

Immediately after a clean pipe comes into contact with water, inorganics, such as calcium, form a conditioning layer. (Fig. 2.3)

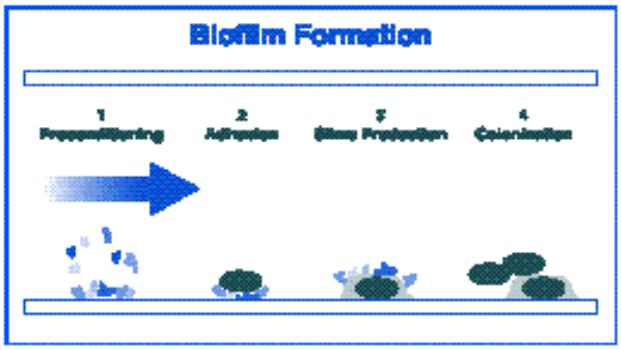

Fig. 2.3: adsorption of organic molecules on a clean surface with following steps: (1) preconditioning (2) adhesion (3) slime productions (4) colonization. (source: Pall Medical 2003)

Step 2: Adhesion of "pioneer" bacteria

Free floating bacteria such as *P. aeruginosa* or *Legionella* will come in contact with the pre-conditioned pipe surface and attach to that surface.

Step 3: Slime formation

"Pioneer" bacteria excrete sticky polymer strands trapping nutrients, protecting from biocides and making up about 75% of the mature biofilm

Step 4: Biofilm slime snares other types of microorganisms and a fully functioning biofilm forms like a "living tissue" on the pipe surface (Pall Medical 2003).

Biofilm was investigated in this study by taking swabs from the interior surfaces of the faucets and shower heads.

Previous Studies

Wellinghausen and others (2001) studied the Contamination of hospital water systems with *Legionella* at three different hospitals belonging to the University of Ulm in Germany between October 2000 and February 2001. A total of 77 potable water samples were collected. The rates of detection of *Legionella* were 70.1% (54 of 77).

Borella and others (2005) studied *Legionella* Contamination in Hot Water of Italian hotels. The hot water systems of Italian hotels were strongly colonized by *Legionella*; 75% of the buildings examined and 60% of the water samples were contaminated, mainly at levels of $\geq 10^3$ CFU/L, and *L. pneumophila* was the most frequently isolated species (87%). *L. pneumophila* serogroup 1 was isolated from 45.8% of the contaminated sites and from 32.5% of the hotels examined. They find that hotel age was associated with contamination.

Borella and others (2004). investigated *Legionella* and *Pseudomonas* contamination of hot water in 146 water samples were collected from private homes of six towns (Milan, Modena, Bologna, Rome, Naples, Bari) representative of different Italian regions (Northern, Central, and Southern Italy). *Legionella* spp. were detected in 33

(22.6%) and *Pseudomonas* spp. in 56 (38.4%) of 146 samples. Some factors associated with *Legionella* contamination were heater type, tank distance and capacity, water plant age, and mineral content. *Legionella* contamination was associated with a centralized heater, distance from the heater point >10 m, and a water plant >10 years old. Furthermore, zinc levels of <100 µg/L and copper levels of >50 µg/L appeared to be protective against *Legionella* colonization.

Leoni and others (2005) , evaluated the prevalence of Legionella species in hot water distribution systems in the city of Bologna (Italy). A total of 137 hot water samples were analyzed: 59 from the same number of private apartments, 46 from 11 hotels and 32 from five hospitals, all using the same water supply. *Legionella* species were detected in 40.0% of the distribution systems, *L. pneumophila* in 33.3%. The highest colonization was found in the hot water systems of hospitals (93.7% of samples positive for *L. pneumophila*, geometric mean: 2.4 * 10^3 CFU/L) followed by the hotels (60.9%, geometric mean: 127.3 CFU/L and the apartments with centralized heating (41. 9%, geometric mean: 30.5 CFU/ L).

Borella and others (2005), investigated *Legionella* spp. contamination of hot water in Italian hotels. The hot water systems of Italian hotels were strongly colonized by *Legionella*; 75% of the buildings examined and 60% of the water samples were contaminated, mainly at levels of $\geq 10^3$ CFU/L, and *L. pneumophila* was the most

frequently isolated species (87%). *L. pneumophila* sg 1 was isolated from 45.8% of the contaminated sites and from 32.5% of the hotels examined.

Barbaree and others (1987), Isolated *L. pneumophila* from two hospitals, the first hospital was an acute care facility in the New England Area with approximately 700 beds and 28 buildings. A total of 12 of 15 legionellosis cases were from one of six main buildings, and all isolates from patients were *L. pneumophila* serogroup 1. *L. pneumophila* were isolated from 43 of 106 sample collected (40%). *L. pneumophila* serogroup 1, 3, 5 were isolated. The second hospital was a northern midwest pediatric hospital with approximately 300 beds. L. pneumophila serogroup 1 were isolated from 13 of 37.

Nosocomial legionnaires'disease (LD) outbreak was occurred in Los Angeles hospital in Los Angeles Country (LAC) in 2002, after this outbreak water sampling and culturing were done for *Legionella* species at all 6 LAC Department of Health Services (DHS) hospital facilities and one country office building, the hall of administration.

Three of six DHS hospital facilities and the one country office building sampled grew *L. pneumophilia* from their water systems. The water supplied of these facilities includes chlorinated water provided by Los Angeles city department of water and power (DWP) (Country of Los Angeles Department of Health Services Public Health 2003).

Doleans and others (2004), made a relationship between hospital water contamination with *Legionella* and hospital-acquired Legionellosis, they examined the level of *Legionella* colonization of hospital water systems in France by studying the 554 water samples predominantly collected from hospitals. The 286 positive water samples (51.6%) contained between 10^2 and 10^7 *Legionella* CFU/L, and 138 samples (48.3% of the positive samples) contained $\geq 10^3$ CFU/L. Despite this frequent contamination of hospital water systems in France, hospital-acquired legionellosis remains relatively infrequent, with about 100 cases annually (15% of all of the cases in France).

Visca and others (1999) reported on an investigation of *L. pneumophila* serogroup 6 isolates from a hospital in northern Italy, in which five sporadic cases of Legionnaires' disease occurred from 1989 to 1997. *L. pneumophila* serogroup 6 was isolated from clinical specimens from two patients, who died, the other three cases of nosocomially acquired Legionnaires' disease were clinically diagnosed in 1994, 1995, and 1997 . An environmental investigation was further performed in 1996. *L. pneumophila* serogroup 6 was isolated from 15 (62.5%) of 24 sites examined. The *Legionella* concentration at the different sites examined ranged from 10^2 to $>10^4$ CFU/L, which is an amount considered to be able to cause one or more sporadic cases per year .

Borella and others (1999) reported, in May 1998, a single case of nosocomial legionellosis was discovered in a 1000 bed hospital in Milan. The hospital's first case of hospital acquired legionnaires' disease was a 29 year old man. He died the next day

and Legionella was identified by immunofluorescence in lung tissue. The environmental surveillance revealed that the centralized hot water distribution system of the hospital was colonised with *L. pneumophila.* Shock heating and hyperchlorination of water was applied, which reduced the number of contaminated sites in the short term, but water was recolonised two months later. During the period of active surveillance from January 1998 to September 1999, six nosocomial cases were identified. In addition, 12 community cases were discovered

Pancer and others (2003), studied the risk of legionellosis due to *L. pneumophila* serogroup1 and serogroup2-14 for patients and hospitals staff members from the water system in a Polish hospital. 32 hot water samples were taken from Warsaw hospital tap water systems. *L. pneumophila* were isolated from 20 of 22 water samples were taken 90% before thermal disinfection (80°C). The hospital water system was heated to 80°C and held at this temperature for 30 min. all distal outlets were flushed with this hot water (80°C). The water system was then maintained to provide water above 55°C at the hot water taps. After thermal disinfection, 40% (4 of 10) samples were contaminated with *L. pneumophila* . It was demonstrated that the high number of *Legionella* in water distribution systems can be successfully reduced by heat treatment from 91-40%.

Bollin and others (1985) made a study about Aerosols Containing *L. pneumophila* generated by shower heads and hot-water faucets. Air was collected above two shower doors and from the same rooms approximately 3 ft (91 cm) from the shower doors while the hot water was running. Low numbers (3 to 5 CFU/15 ft^3 [0.43 m^3] of air) of *L. pneumophila* were recovered above both shower doors, but none was

recovered from the air in either room outside the shower door. Approximately 90% (7 of 8 CFU) of the *L. pneumophila* recovered were trapped in aerosol particles between 1 and 5 μm in diameter. Air was collected 1 to 3 ft (30 to 91 cm) from 14 sinks while the hot water was running. Low numbers (1 to 5 CFU/15 ft^3 of air) were recovered from 6 of 19 air samples obtained. Approximately 50% (6 of 13 CFU) of the organisms recovered were trapped in aerosol particles between 1 and 8, μm in diameter. Shower heads and hot-water taps containing L. pneumophila can aerosolize low numbers of the organism during routine use. The aerosol particle size is small enough to penetrate to the lower human respiratory system. Thus, these sites may be implicated as a means of transmission of L. pneumophila from potable water to the patient.

Prevalence of Legionellosis Disease among European Countries

Carol J. Kate R. (2005) reported on behalf of the European Working Group for *Legionella* Infections that In Europe the aggregated annual totals of legionellosis have increased from 1161 in 1994 to 4578 in 2003, with the majority of the rise taking place since 2000. Aggregated incidence rates per million populations have increased from 3.35 in 1994 to 9.8 in 2003. In 2003, 34 countries submitted an annual dataset and individual country rates ranged from 0.0 (Latvia, Lithuania) to 28.7 (Spain) per million population.

Table 2.1 shows the rate of legionnaires' disease in European countries in 1999.

Table 2.1: Rate of legionnaires' disease in European countries in 1999

Country	Rate per million population
Belgium	19.5
Denmark	16.98
The Netherlands	16.75
Switzerland	1075
Sweden	9.71
Malta	7.9
Spain	7.76
France	7.6
Scotland	6.81
Austria	5.13
Italy	4.05
England & Wales	3.72
Northern Ireland	2.94
Norway	2.27
Finland	1.76
Ireland	0.6

source: O'Flanagan and others (2002)

Stella and others (2005) reported on behalf of the European Working Group for *Legionella* Infections that In Europe study was for estimation the risk of legionnaire`s disease in hospitals of Northern and Central Greece by isolating *L. pneumophila*. They collected 189 water samples from ten hospitals during 2004. *L. pneumophila* was isolated in 29 samples from six out of ten hospitals.

L. pneumophila serogroup 1 was isolated in 11 samples obtained from five hospitals in concentrations ranging from 1000 to 100000 CFU/L. *L. pneumophila* strains serogroups2-14 were isolated in 16 samples obtained from 5 hospitals. Most of the serogroups2-14 strains were isolated from rooms where high risk patients were hospitalized (ICUs, Bone marrow transplantation units, Immunodeficient patients Units).

Attar and others (2004) Identified of Legionella in the Hot Water Supply of a General Hospital in Isfahan, they isolated Legionella from 11 of the total 30 samples (36.6%). They reported that many outbreaks of Legionnaire's disease have been reported in different parts of the world, Annually 8,000 to 18,000 cases of the Legionnaire's disease are reported in USA, of which 10 to 20% are the cases reported during epidemics with a death toll of about 20 to 40 percent. Hospitals in different countries around the globe have been surveyed for Legionella contamination. The results of the surveys in UK revealed that the water distribution systems in 70% of the 40 studied hospitals contained Legionella growths. This is while in one study 60% of the studied hospitals in western Pennsylvania (15 hospitals), 23% in Scotland (39 hospitals), 68% in Quebec, Canada (84 hospitals), and in another study 12% of the hospitals in UK (out of 17 hospitals) were found to be Legionella positive.

Nosocomial infection by *Pseudomonas aeruginosa*

Giamarellou (2002) mentioned that *P. aeruginosa* is among the most common pathogens involved in nosocomial infections. In the European Prevalence of Iinfection in Intensive Care (EPIC) study, *P. aeruginosa* was recognized as the predominant Gram-negative species (28.7%) isolated from bronchopulmonary infection sites of patients hospitalized in 1417 intensive care units (ICUs) of 17 Western European countries.

According to the National Nosocomial infections Surveillance (NNIS) study, out of 39810 isolates collected in the USA between 1990 and 1999, *P. aeruginosa* predominated (17%) among Gram-negatives associated with hospital-acquired ICU pneumonia. In the most recent SENTRY study, performed in 1997 in Canada, USA and Latin America, among 4267 blood stream isolates *P. aeruginosa* was the third most common pathogen (10.6%).

Trautman and others (2001) studied tap water colonization with *P. aeruginosa* in a surgical intensive care unit. Twenty-nine percent (5 out of 17) patients were infected with a genotype identical to that found in the tap water samples. All water outlets harbored distinct genotypes of *P. aeruginosa* over prolonged periods of time.

Kolmos and others (1993) identified showers and the tubing connecting the showerhead to the tap as a source of transmission of *P. aeruginosa*. This led to septicemia in five patients as a result of using contaminated shower equipment to irrigate burn wounds.

Anaissie and others (2003) estimated that 1,400 deaths occur each year in hospitals in the United States as a result of waterborne nosocomial pneumonia caused by *P. aeruginosa* alone with no clear guidelines for prevention of these infections. They presumed that the primary cause of diminished water quality as the buildup of biofilm and the corrosion of distribution lines and tank surfaces in the hospital.

Brito and others (2003) talked in their study about outbreaks of multiresistant *P. aeruginosa* infection in Newborn Intensive Care Units (NICU) are often associated with high mortality rates. Preterm infants are potentially at risk for infection with *P. aeruginosa* because they may be immunocompromised, they often require supplemental oxygen and/or mechanical ventilation, and they require prolonged hospitalization. *P. aeruginosa* conjunctivitis during infancy may lead to a rapidly progressive, invasive eye infection. In some cases this destructive eye disease is associated with or followed by infection at other sites, but there have been cases of *P. aeruginosa* conjunctivitis in hospitalized, premature infants who developed systemic complications without invasive eye infection. They reported an outbreak of conjunctivitis due to *P. aeruginosa* involving seven infants admitted in the Neonatal Intensive Care Unit (NICU) of the Uberlândial Federal University Hospital between March and September 2001. Ten isolates were obtained from conjunctival cultures. One of the infants, who was born prematurely and weighed less than 1000g, subsequently developed a fulminating septicaemia, also provoked by *P. aeruginosa*; this patient died. This epidemic strain of *P. aeruginosa*, could have been disseminated by health care worker's hands or by aerosolization from endotracheal tube aspirates of the respiratory tract of neonates requiring prolonged endotracheal intubation and mechanical ventilation; the epidemic strain of *P. aeruginosa* was isolated from the respiratory tract of two infants (cases 1 and 6), and later, after infection was diagnosed in their eyes, developed pneumonia by clinical and radiological criteria. Among these seven hospitalized infants with *P. aeruginosa* conjunctivitis, only three (1 sepsis and 2 pneumonias) developed systemic complications of *P. aeruginosa* infection; in the

other four there was no evidence of invasive eye disease, probably due to early detection and treatment with eye drops.

Chapter Three

Material and methods

From May through August 2005, a total of 134 water samples were collected (53 samples for *Legionella* analysis, and 81 samples for *P. aeruginosa*) from six governmental hospitals of West Bank - Ramallah, Alia- Hebron, Beit Jalla, Alwatani, Rafidia, and Jenin – representative of different regions of West Bank (Northern, Central, and Southern West Bank). Samples were taken from each hospital randomly.

The samples were collected with helping of Environmental Health Department - Ministry Of Health (MOH). The samples were collected according to international standards. Samples testing were carried out in the Central Public Health Laboratory (CPHL) of MOH in Ramallah.

The following describe the procedures for the *Legionella* and *P. aeruginosa* analysis:

Legionella

Sample collection

Hot water samples (42- 50°C) were drawn from the bathroom outlets (shower head or bathroom tap) in sterile 1L glass bottles after a brief flow time, 2-3 min, to eliminate cold water inside the tap and to permit clearing the service line. Water flow was

reduced to permit filling bottle without splashing. To neutralize residual free chlorine, sodium thiosulphate was added in sterile bottles for bacteriologic analysis (American Public Health Association APHA 1998; Borella *et al* 2004).

In addition to collecting water samples, swab samples for collecting biofilm on the interior surfaces in the faucets and showerheads was taken. The swab samples resuspended and mixed with the water samples (American Public Health Association APHA 1998; Yang 2004).

Collection bottles were returned to the laboratory immediately after sampling for examination, if analysis would not begin within 24 hours, samples were kept at >4°C and processed within 48 hours of collection (Borella *et al* 2004).

Legionella analysis

To detect *Legionella* spp.

1. 1-L water samples were concentrated by membrane filtration (0.45-µm-pore–sized cellulose membrane filters, 47 mm in diameter) (Bartie *et al* 2001).

2. The filter membrane was resuspended in 10 mL of original sample water in sterile bag and vortex-mixed for 10 min (Borella *et al* 2004; Leoni *et al* 2005).

3. Two aliquots of 0.1 mL of the original and concentrated specimens 1:10 diluted with sterile tryptone water and undiluted were each spread on buffered

charcoal yeast extract (BCYE) agar (Oxoid CM0655) with BCYE growth supplement (Oxoid SR0110A) (ACES buffer/ potassium hydroxide, L-cysteine, ferric pyrophosphate, α- ketoglutarate)and MWY selective supplement (Oxoid SR0118E) (anisomycin, glycine, polymyxin B, vancomycin, bromocresol purple and bromothymol blue [Australian standard AS method (AS3896-1991)] (Bartie *et al* 2001).

4. The plates were incubated at 37°C in a humidified aerobically environment for 10 days and read from day 3 (Bartie *et al* 2001; Borella *et al* 2004; Leoni *et al* 2005).

5. Suspected colonies with a mottled surface or the typical ground glass appearance of *Legionella* species (see Fig.3.1) , were counted from each sampling, 3-6 random colonies from each plate were subcultured on (BCYE) agar (with cysteine) and blood agar (cysteine-free) media for ≥2 days (Alyssa *et al* 1995; Leoni *et al* 2005; Brown *et al* 1982).

Fig.3.1: *Legionella* bacteria grown on MWY medium (Vos MC., Troelstra A. 2001).

6. *L . pneumophila* grew on BCYE agar but fail to grow on blood agar, it grew as a bleu-gray. Only colonies grown on BCYE were subsequently identified by an agglutination test (*Legionella* Latex Test, Oxoid DR0800M) ((Bartie *et al* 2001; Leoni *et al* 2005).

7. The test allows a separate identification of *L. pneumophila* serogroup 1 and serogroups 2–14 and detection of seven *Legionella* (polyvalent) species (other than *L. pneumophila*), which have been implicated in human disease.

8. The results are expressed as CFU/L.

9. Biofilm analysis
    1. mix the tube containing cotton swab with agitator for 2 minutes
    2. spread 0.1 ml of the above tube on MWY agar plate
    3. The plates were incubated at 37°C in a humidified aerobically environment for 10 days and read from day 3 (American Public Health Association APHA 1998; Yang 2004).

Pseudomonas aeruginosa

Sample collection

Water samples were drawn from the bathroom outlets (shower head or bathroom tap) in sterile 250ml glass bottles, after a brief flow time, 2-3 min, or for time sufficient to permit clearing the service line, water flow is reduced to permit filling bottle without

splashing. Leave small air space in the bottle to facilitate mixing by shaking (American Public Health Association APHA 1998).

Collection bottles were returned to the laboratory immediately after sampling, samples were kept at <10°C, the time elapsing between collection and examination should not exceed 24 hours (American Public Health Association APHA 1998).

Pseudomonas aeruginosa analysis

To isolate *Pseudomonas aeruginosa*:

1. 200ml water samples were filtered through a 0.45-µm filter membranes (0.45-µm -pore-sized cellulose membrane, 47mm in diameter) (American Public Health Association APHA 1998; Bartie *et al* 2001).
2. The membranes were placed on Cetrimide agar (HIMEDIA M024) plate without air space between the membrane and the agar surface. Incubate at 37°C for 24-48 hours (Borella *et al* 2004; United States Pharmacopoeia 2002).
3. Typically *P. aeruginosa* colonies are 0.8 to 2.2 mm in diameter and flat in appearance with light outer rims and brownish to green –black centers. Count the number of greenish colony (American Public Health Association APHA 1998).
4. To identify suspected colonies make oxidase test (Abtek), (*P. aeruginosa* oxidase- positive) . a positive test is indicated by the development of a dark purple color. No color development indicated a negative test and the absence

of the enzyme. The oxidase test determines the presence of oxidase enzymes (American Public Health Association APHA 1998).

5. Report the result per 200 ml (American Public Health Association APHA 1998).

Quality control for *Legionella*

The important step is done in the project to make sure that our isolation is certified, by culture of American Type Culture Collection (ATCC 33156) L. *pneumophila* serogroup 4, on MWY , and incubate the plates at 37°C in a humidified aerobically environment for 10 days and read from day 3. Suspected colonies with the typical ground glass appearance of *Legionella* species , were subcultured on (BCYE) agar (with cysteine) and blood agar (cysteine-free) media for ≥2 days.

L . pneumophila grew on BCYE agar but fail to grow on blood agar, it grew as a bleu-gray. Only colonies grown on BCYE were subsequently identified by an agglutination test (*Legionella* Latex Test, Oxoid DR0800M) (Bartie *et al* 2001).

The results appear that isolation is *Legionella pneumophila* sg (2–14) because the test allows identification of *L. pneumophila* serogroup 1 and sg (2–14) but cant identified *Legionella pneumophila* serogroup 4 with alone.

Heat Disinfection for *Legionella* and *Pseudomonas aeruginosa* Control

The hospital water system was heated to 80°C in Beit Jallah hospital and 70 °C in Jenin hospital and held at this temperature for 30 minute at least. All distal outlets were flushed with this hot water .the samples were collected before and after heat disinfection to demonstrate the effect of heat on microorganisms.

Chapter four

Results

Legionella Bacteria

L. pneumophila sg (2-14) was isolated from 33 (62.3%) of 53 samples that were analyzed. from West Bank hospitals. In the positive samples, the mean number of *L. pneumophila* was 6.17 x 10^3 CFU/L (range 100 CFU/L to 2.85 x 10^4 CFU/L); Table 4.1 summarizes the distribution of samples according to the hospitals, and brief results of *Legionella* analysis.

Table 4.1: *Legionella* analysis of water samples collected from West Bank hospitals

Hospital	No. & (%) of samples	No. & (%) Contaminated samples	No. & (%) Uncontaminated samples
Ramallah	16 (30)	2 (12.5)	14 (87.5)
Alia- Hebron	18 (34)	13 (72.2)	5 (27.8)
Beit Jalla	8 (15)	8 (100)	0 (0)
Alwatani- Nablus	3 (6)	3 (100)	0 (0)
Rafidia- Nablus	3 (6)	2 (66.6)	1 (33.4)
Jenin	5 (9)	5 (100)	0 (0)
Total	53 (100)	33 (62.3)	20 (37.7)

Contamination of West Bank Hospitals water with *Legionella*

Table 4.2 shows the results of West Bank hospitals samples. Samples were collected from different divisions of hospitals. 16 samples were collected from Ramallah hospital two samples were contaminated with *L. pneumophila* sg (2-14). 18 samples were collected from Alia- Hebron hospital, *L. pneumophila* sg (2-14) were isolated from 13 (72%) samples, the mean number of *L. pneumophila* sg (2-14) was $6.5*10^3$ CFU/L, with range from 100 CFU/L to $1.65*10^4$ CFU/L . 8 samples were collected from Beit Jallah hospital, all samples were contaminated with *L. pneumophila* sg (2-14), with range $1.2*10^3$CFU/L to $6.5*10^3$ CFU/L. 3 samples were collected from Al-Wtani hospital, all samples were contaminated with *L. pneumophila* sg (2-14), with mean 700 CFU/L and range between 200 CFU/L to $1.4*10^3$ CFU/L. 3 samples were collected from Rafidia hospital, two of them were contaminated with *L. pneumophila* sg (2-14). 5 samples were collected from Jenin hospital all samples were contaminated with *L. pneumophila* sg (2-14), with mean $1.6*10^4$ CFU/L, the concentration of *Legionella* sg (2-14) varied from $3.8*10^3$ CFU/L to $2.85*10^4$ CFU/L.

Table 4.2: *Legionella* Results of West Bank hospitals

Hospitals	Division	Source	No. & (%) of distribution samples	No. & (%) of positive samples
Ramallah	Pediatric	Shower & Faucet	7 (43.75)	1 (6.25)
	Surgery	Shower & Faucet	7 (43.75)	1 (6.25)
	Women	Shower	1 (6.25)	0 (0)
	Delivery	Shower	1 (6.25)	0 (0)
Total			16 (100)	2 (12.5)
Hebron	Pediatric	Shower & Faucet	6 (33.3)	6 (33.3)
	Surgery	Shower & Faucet	5 (27.8)	4 (22.2)
	ICU	Shower & Faucet	2 (11.1)	2 (11.1)
	Delivery	Shower & Faucet	4 (22.2)	1 (5.6)
	Physical Medician Rehabilitation	Shower	1 (5.6)	0 (0)
Total			18 (100)	13(72.2)
Beit Jallah	Pediatric	Shower	4(50)	4 (50)
	Surgery	Shower	2 (25)	2 (25)
	Delivery	Shower	2 (25)	2 (25)
Total			8 (100)	8(100)
Al-Wtani	Pediatric	Shower	3 (100)	3 (100)
Rafidia	Incubator	Shower	1 (33.3)	0(0)
	Delivery	Shower	1 (33.3)	1 (33.3)
	Burn	Shower	1 (33.3)	1 (33.3)
Total			3 (100)	2 (66.6)
Jenin	incubator	Shower	1 (20)	1 (20)
	surgery	Shower	1 (20)	1 (20)
	delivery	Shower	1 (20)	1 (20)
	women	Shower	1 (20)	1 (20)
	ICU	Shower	1 (20)	1 (20)
Total			5 (100)	5 (100)

Total			53 (100)	33 (62.3)

Main Water Storage Reservoirs of Rafidia and Al-Watani (Nablus) hospitals

Two water samples were collected from the storage reservoirs of Rafidia and Al-Watani (Nablus) hospitals, they are uncontaminated with *Legionella* , whereas the water from the distribution systems of two hospitals were contaminated with *L. pneumophila* sg (2-14), this indicates the contamination is from the biofilm available on the interior surfaces of water distribution systems.

Thermal disinfection on *Legionella*

The hospital water was heated to 80°C in Beit Jallah hospital and 70°C in Jenin hospital to demonstrate the *Legionella* colonization before and after thermal disinfection.

Beit Jallah hospital

Before thermal disinfection, 8 samples were collected from Beit Jallah hospital, all samples were contaminated with *L. pneumophila* sg (2-14), with concentration varied from $1.2*10^3$ CFU/L to $6.5*10^3$ CFU/L (Table 4.3), after thermal disinfection at 80°C, 6 samples were collected. *L. pneumophila* sg (2-14) were detected in one sample only with 200 CFU/L (Table 4.4)

Table 4.3: *Legionella* Results of Beit Jallah hospital before thermal disinfection

	Division	Source	Result
1	Pediatric	Shower	$3*10^3$ CFU/L (L.p. 2-14)
2	Pediatric	Shower	$2.1*10^3$CFU/L (L.p. 2-14)
3	Pediatric	Shower	$4.4*10^3$CFU/L (L.p. 2-14)
4	Surgery	Shower	$1.2*10^3$CFU/L Biofilm:2CFU (L.p. 2-14)
5	Surgery	Shower	$3.6*10^3$CFU/L Biofilm:1CFU (L.p. 2-14)
6	Delivery	Shower	$3.3*10^3$CFU/L Biofilm:4CFU (L.p. 2-14)
7	Delivery	Shower	$6.2*10^3$ CFU/L Biofilm:3CFU (L.p. 2-14)
8	Pediatric	Shower	$6.5*10^3$ CFU/L (L.p. 2-14)

Table 4.4: *Legionella* Results of Beit Jallah hospital (after thermal disinfection at 80°C)

	Division	Source	Rresults
1	Delivery	Shower	200 CFU/L (L.p. 2-14)
2	Surgery	Shower	Nil
3	Surgery	Shower	Nil
4	Tumors	Shower	Nil
5	Pediatric	Shower	Nil
6	Emergency	Shower	Nil

Jenin hospital

In Jenin hospital before thermal disinfection, 5 samples were examined for *Legionella* analysis, all samples were contaminated with *L. pneumophila* sg (2-14), with concentration varied from 3.8*$*10^3$* CFU/L to 2.85*$*10^4$* CFU/L (Table 4.5), after thermal disinfection at 70°C, 5 samples were collected. *L. pneumophila* sg (2-14) were detected from all samples, but with less concentration, the range become from 1.9*$*10^3$* CFU/L to 1*$*10^4$* CFU/L (Table 4.6)

Table 4.5: *Legionella* Results of Jenin hospital
(before thermal disinfection)

	Division	Source	Results
1	Delivery	Shower	3.8*10^3CFU/L (L.p. 2-14)
2	Women	Shower	2.85*10^4CFU/L (L.p. 2-14)
3	Incubator	Shower	1.44*10^4 CFU/L (L.p. 2-14)
4	ICU	Shower	2.15*10^4 CFU/L (L.p. 2-14)
5	Surgery	Shower	1.16*10^4 CFU/L (L.p. 2-14)

Table 4.6: *Legionella* Results of Jenin hospital
(after thermal disinfection at 70°C)

	Division	Source	Results
1	Delivery	Shower	1.9*10^3CFU/L (L.p. 2-14)
2	Women	Shower	2.3*10^3CFU/L (L.p. 2-14)
3	Incubator	Shower	1.0*10^4 CFU/L (L.p. 2-14)
4	ICU	Shower	4.5*10^3 CFU/L (L.p. 2-14)
5	Men	Shower	8.4*10^3 CFU/L (L.p. 2-14)

Pseudomonas Aeruginosa Bacteria

P. aeruginosa were isolated from 17 of 81 (21%) samples, from West Bank hospitals, two hospitals were contaminated with *P. aeruginosa*: Ramallah hospital, and Jenin hospital, with levels ranging from 1 to TNTC CFU/200 ml. (Table 4.7)

Table 4.7: Results of samples collected for *P. aeruginosa* analysis from West Bank hospitals

Hospital	No. & (%) of Distribution samples	No. & (%) Contaminated samples	No. & (%) Uncontaminated samples
Ramallah	24 (30)	7 (29)	17 (71)
Alia- Hebron	18 (22)	0 (0)	18 (100)
Beit Jalla	13 (16)	0 (0)	13 (100)
Alwatani- Nables	5 (6)	0 (0)	5 (100)
Rafidia- Nables	11 (14)	0 (0)	11 (100)
Jenin	10 (12)	10 (100)	0 (0)
Total	81 (100)	17 (21)	64 (79)

Contamination of West Bank Hospitals water with *P.aeruginosa*

Table 4.8 shows the *P. aeruginosa* Results of water samples collected from West Bank hospitals, samples were collected from different divisions of hospitals. Ramallah and Jenin hospitals only were contaminated with *P. aeruginosa.* Whereas all samples collected from the others hospitals were negative. 24 samples were collected from Ramallah hospital, 7 samples were collected from Pediatric Division were contaminated with *P. aeruginosa*, with concentration range between 1 CFU/200ml to 45 CFU/200ml. 10 samples were collected from Jenin hospital for *P.aeruginosa* analysis all samples were contaminated with *P. aeruginosa* , with range varied from 15 CFU/200ml to TNTC CFU/200ml.

Table 4.8: *P. aeruginosa* Results of West Bank Hospitals

Hospitals	Division	Source	No. & (%) of distribution samples	No. & (%) of positive samples
Ramallah	Pediatric	Shower & Faucet	7 (29)	7 (100)
	Surgery	Shower & Faucet	10 (41)	0 (0)
	Delivery	Faucet	4 (17)	0 (0)
	Women	Faucet	3 (13)	0 (0)
Total			24 (100)	7 (29.2)
Alia	Pediatric	Faucet	8 (42)	0 (0)
	Surgery	Faucet	4 (21)	0 (0)
	Delivery	Faucet	5 (26)	0 (0)
	ICU	Faucet	1 (11)	0 (0)
Total			18 (100)	0 (0)
Beit Jallah	Pediatric	Faucet	7 (54)	0 (0)
	Surgery	Faucet	3 (23)	0 (0)
	Delivery	Faucet	3 (23)	0 (0)
Total			13 (100)	0 (0)
Al-Wtani	Pediatric	Faucet	5	0
Rafidia	Pediatric	Faucet	1 (9)	0 (0)
	Surgery	Faucet	5 (46)	0 (0)
	Delivery	Faucet	3 (27)	0 (0)
	Burns	Faucet	2 (18)	0 (0)
Total			11 (100)	0 (0)
Jenin	Pediatric	Faucet	4 (40)	4 (40)
	Surgery	Faucet	1 (10)	1 (10)
	Delivery	Faucet	3 (30)	3 (30)
	Womens	Faucet	2 (20)	2 (20)
Total			10 (100)	10 (100)
Total			81 (100)	17 (21)

Thermal disinfection on *Pseudomonas Aeruginosa*

Jenin hospital

Before thermal disinfection at 70°C, 10 samples were collected from Jenin hospital, all samples were contaminated with *P. aeruginosa*, (Table 4.9), after thermal disinfection at 70°C, 5 samples were collected. all samples were negative for *P. aeruginosa*, (Table 4.10).

Table 4.9: *P. aeruginosa* Results of Jenin hospital

(before thermal disinfection)

	Division	Source	Results
1	Pediatric	Faucet	TNTC
2	Pediatric	Faucet	TNTC
3	Pediatric	Faucet	TNTC
4	Delivery	Faucet	TNTC
5	Delivery	Faucet	TNTC
6	Delivery	Faucet	15CFU/200ml
7	Women	Faucet	TNTC
8	Women	Faucet	17CFU/200ml
9	Incubator	Faucet	31CFU/200ml
10	Surgery	Faucet	TNTC

Table 4.10: *P. aeruginosa* Results of Jenin hospital
(after thermal disinfection at 70°C)

	Division	Source	Results
1	Incubator	Faucet	Nil
2	Delivery	Faucet	Nil
3	Men	Faucet	Nil
4	ICU	Faucet	Nil
5	Women	Faucet	Nil

Chapter five

Discussion

In this study *Legionella* spp. were isolated 62.3%. from hot water samples collected from West Bank hospitals. The mean number of *Legionella* in the positive samples was 6.17 x 10^3 CFU/L (range 100 CFU/L to 2.85 x 10^4 CFU/L). In previous studies, in Germany, the contamination of hospitals water systems were similar 70.1% (Wellinghausen *et al* 2001). In UK 70% of hospitals were contaminated with *Legionella* growth, 60% of the studied hospitals in Western Pennsylvania, 23% in Scotland, 68% in Quebec Canada, 36.6% in Isfahan (Attar *et al* 2004).

This study concerned about hot water systems of hospitals, whereas *Legionella* can prevalence in domestic and hotels water systems also. Leoni and others 2005, made detection of *Legionella* species in domestic, hotels, and hospitals hot water systems, their results were 93.7% of hospitals, 60.9% of hotels, and 41.9% of domestic water systems were contaminated with *Legionella* species. Another Italian study of hot water samples taken from domestic water system, *Legionella* were isolated from 22.6%, and *P. aeruginosa* isolated from 38.4% (Borella *et al* 2004).

In this study *L. pneumophila* sg (2-14) was predominant, according to survey in Italy *L. pneumophila* is by far the most abundant species in potable and environmental

water samples, as >75% of positive samples were contaminated by *L. pneumophila* (Borella *et al* 2004). This study showed that in West Bank hospitals all of positive samples were contaminated by *L. pneumophila* sg (2-14).

L. pneumophila and occasionally other *Legionella* have also been reported to cause disease. *L. pneumophila* is the most pathogenic of the species, causing up to 90% of the cases of legionellosis (World Health Organization WHO 1996; Yang 2004). Unfortunately, there are no previous data about legionellosis cases in Palestine.

The study was not designed specifically to address the epidemiologic link between showering and acquisition of nosocomial Legionnaires disease. We only wished to determine if water systems in West Bank hospitals are contaminated with *Legionella* and *P. aeruginosa* to give the decision maker a clear view about situation and to promote for new surveillance about link between logionellosis and water contamination with *Legionella*.

The *P. aeruginosa*, on the other hand were found 21% of water samples it less prevalence than *L. pneumophila* sg (2-14) , according to a survey in Italy (Leoni et al 2005) *P. aeruginosa* were found but nearly always at low concentrations and limited to single sampling point rather than affecting the whole system.

Pancer and others 2003 have demonstrated that the growth of *Legionella* can be reduced by high water temperatures up to 70°C, in their study *L. pneumophila* sg 1 was eliminated after heat treatment at 80°C for 30 minute, while *L. pneumophila* sg

(2-14) was detected, however the concentration decreased by considerable amount for example from $1.0*10^4$CFU/L to 2710^4CFU/L . In this study, the hospital water was heated to 80°C in Beit Jallah hospital and 70°C in Jenin hospital for 30 minutes, to demonstrate the *Legionella* colonization before and after thermal disinfection. In Biet Jallah hospital after thermal disinfection at 80°C , *L. pneumophila* positive samples were reduced from 100% before heat disinfection to 17% after heat disinfection. While at Jenin hospital thermal disinfection (70°C) reduced the number of *L. pneumophil*a colonization but not completely eliminated it. It was demonstrated that the high number of *L. pneumophila* in water distribution systems can be successfully reduced by heat treatment.

In comparing the results after thermal disinfection between Beit Jallah hospital and Jenin hospital, it was noticed that, the thermal disinfection in Beit Jallah hospital is more effective than in Jenin hospital, as the temperature in Beit Jallah hospital reached 80°C while in Jenin hospital it reached to 70°C, that's indicate that the aggregation of biobilm entire water distribution system in Jenin hospital need higher temperature and longer time period for killing bacteria.

Thermal disinfection at 70°C in Jenin hospital can be successfully efficient to eliminated high number of *P. aeruginosa* in water distribution systems. The *P. aeruginosa* positive samples reduced from 100% before heat disinfection to 0% after heat disinfection.

Two water samples from main water reservoir of Rafidia and Al-Watani (Nablus) hospitals were analyzed for *Legionella* , they are uncontaminated, whereas the water from the distribution systems of two hospitals were contaminated, this indicates the contamination is from the biofilm available on the interior surfaces of water distribution systems.

To prevent the accumulation of microbial sludge and slime – biofilm- , a factor known to be associated with outbreaks of legionnaires' disease LD, hospital water systems should be cleaned regularly including showerhead , faucets and hot water storage tank (Pancer *et al* 2003).

CDC recommended that the hospital water should filtered by 0.2 μm filter which can be placed easily on faucets and showerheads, and prevent bacteria pass through it. Chlorination of water to achieve 1-2 ppm of free residual chlorine at the tap, especially in areas where immunosuppressed and other high risk patients are located.

Additional preventive measures have been recommended by CDC to protect severely immunosuppressed patients were restricted from taking showers, and for these patients only sterile water used (Centers for Disease Control and Prevention CDC 1997)

Association between age of distribution system and % of contamination

Table 5.1 shows the association between age of distribution system and % of contamination in West Bank hospitals, as mentioned earlier the primary cause of poor water quality is the build-up of biofilm entire surface of water distribution systems and tank, *p. aeruginosa and Legionella* are often considered from pioneers in creating the biofilm seen in water distribution system, with time the accumulation of biofilm increase and the elimination of these bacteria become difficult due to nature of these bacteria in tolerance to wide variety of physical conditions, including temperature and chlorination.

It was noticed that Jenin water system is considerably new. However, it was highly contaminated, after inquiry from maintenance department it turned out that there is a problem in the control of water temperature, it couldn't be raised more than 50°C, which is as mentioned earlier, it is a very suitable environment for living and the production of this bacteria within the hospitals water system.

This situation calls for the promote application of decontamination measures, especially in hospitals, although the efficiency of such action cannot always be guaranteed considering that *Legionella* may be protected from disinfection due to their growing within ameba and biofilm. It is imperative, therefore, to identify and act on the factors leading to the development of biofilm, and thus *Legionella* in the water network

Table 5.1: Relationship between Age of distribution system and % of contamination in West Bank Hospitals

Hospital	Department	Age of distribution system	No. & % of contaminated with *Legionella*	No. & % of contaminated with *P. aeruginosa*
Ramallah	Main distribution (external)	>15 years	2 (12.5)	7 (29)
	All departments	During two years		
Alia- Hebron	Surgery, children, ICU, incubator	>17 years	13 (72.2)	0 (0)
	Delivery, Emergency	2 years		
Beit Jalla	All departments	10 years	8 (100)	0 (0)
Rafidia-Nables	All departments	28 years	2 (66.6)	0 (0)
Jenin	Delivery, women, men, ICU	6 years	5 (100)	100 (100)
	Surgery, Emergency	3 years		

Contamination of Pediatric Division in West Bank hospitals

Children are the group with higher risk to have waterborne nosocomial infection. In this study it was noticed that all Pediatric Division in West Bank hospitals were contaminated with either P. aeruginosa or *L. pneumophila* or both, which is considered a real health hazard to children's health, also the faucet in incubators room where premature babies bathed in are contaminated which cause morbidity and mortality for babies. (Table 5.2)

Table 5.2: contamination of pediatric division in West Bank hospitals No.& %

Hospital	Room	*Legionella*		*P. aeruginosa*	
		No. samples	No.& % contaminated	No. samples	No.& % contaminated
Ramallah	Patients room	6	0 (0)	6	6 (100)
	Incubators	1	0 (0)	1	1 (100)
Alia-Hebron	Patients room	5	5 (100)	6	0 (0)
	Incubators	1	1 (100)	2	0 (0)
Beit Jalla	Patients room	4	4 (100)	7	0 (0)
Alwatani-Nables	Patients room	2	2 (100)	4	0 (0)
	Incubators	1	1 (100)	1	0 (0)
Rafidia-Nables	Incubators	1	0 (0)	1	0 (0)
Jenin	Patients room	0	0 (0)	3	3 (100)
	Incubators	1	1(100)	1	1 (100)
Total		22 (100)	14 (63.6)	32 (100)	10 (31.3)

Hospitals who care for immunocompromised patients should maintain high standards of water quality and should take immediate measures to prevent waterborne infections. Such measures are likely to be successful, given the large reductions in waterborne infections observed when these guidelines are applied.

Chapter Six

Conclusion and Recommendations

Large numbers of *Legionella* in water distribution systems present a potentially serious health risk to workers and the public.

L. pneumophila sg (2-14) was isolated from 33 of 53 (62.3%) water samples from West Bank hospitals were analyzed.

Legionnaires' disease is normally acquired by inhalation or aspiration of *Legionella* from a contaminated environmental source. *Legionella* concentrations of 3–7,000 CFU/L could be sufficient to produce one case per year in a susceptible population (Borella *et al* 2004), and these contamination levels correspond to those found in our study at the hospital water system

P. aeruginosa is among the most common pathogens involved in nosocomial infections. (Giamarellou 2002) In the United States 1,400 deaths occur each year in hospitals as a result of waterborne nosocomial pneumonias caused by *P. aeruginosa* alone (Anaissie et al. 2003)**.** In our study *P. aeruginosa* were isolated from 17 of 81 (21%) samples, from West Bank hospitals.

This clearly illustrates the importance of protecting the hospital water from contamination with pathogenic bacteria and highlights the need for appropriate specific guidelines for protecting patients from exposure.

Children are the most valuable group of our community , this research is a trial to reduce their morbidity and mortality caused by nosocomial infection due to *P. aeruginosa and Legionella*. The results of the study shows that, the hospitals water are contaminated with dangerous opportunistic pathogens, in Pediatric Division in West Bank hospitals, which endangers the children's health and risk their lives.

Recommendations

To reduce the possibility that *Legionella* species may amplify in the potable water system:

3. The routine periodic culturing of water samples from the hospital's potable water system for the purpose of detecting *Legionella* species, and determination whether there is contamination and whether remediation is necessary or not.

4. Hot water should maintenance above or equal 50° (according to CDC recommendations)
5. Maintain hot water free chlorine residual at 1-2 mg/L at the tap

6. The super-heating method should be done periodically in all hospitals, heat disinfection at Jenin hospital should repeated by raising the hot temperature to at least 80ºC and for longer time > 30minute. The hot water is to circulate and flush the entire water system and the outlets for a period of time. Temperature and duration of flushing depending upon the age of the plumbing system and the thickness of accumulated biofilm

7. Regularly clean showerhead and faucet aerators.

8. Regularly clean hot water storage tank, and equipment that filters potable water such as endoscope preprocessors.

9. Using filters that remove bacteria. The CDC and Prevention recommends that the water filters have 0.2 micrometer filtration capability. Filters are successful in filtering bacteria and can be placed easily on faucets and showers.

10. Medical equipment should be raised with sterile water, because tap or locally prepared distilled water might contain microorganisms that can cause pneumonia

Additional preventive measures should be used to protect patients

1. Immunosuppressed patients to be restricted from taking showers, and, for these patients, only sterile water to be used for drinking or flushing nasogastric tubes
2. Neonatal should be bathed with sterile water

3. Patients with burn should not be exposed their wounds to the tap water only use sterile water

More researches should be done to prevent L*egionella* from prevalence:

1. There is insufficient information to fully understand the link between water colonization and infection. So we recommend heightened surveillance for nosocomial infections on all hospital wards.

2. An evaluation of prevalence of *Legionella* in domestic, hotels, and private hospital should be done

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Annexes

Annex1: Tables

Table A1.1: *Legionella* Results of West Bank hospitals

Hospital		Division	Source	Results
Ramallah	1	Pediatric	Shower	$6.6*10^3$CFU/L (L.p. 2-14)
	2	Pediatric	Shower	Nil
	3	Pediatric	Shower	Nil
	4	Pediatric	Shower	Nil
	5	Pediatric	Faucet	Nil
	6	Pediatric	Faucet	Nil
	7	Incubator	Faucet	Nil
	8	Surgery	Shower	Nil
	9	Surgery	Faucet	Nil
	10	Surgery	Shower	Nil
	11	Surgery	Faucet	200 CFU/L (L.p. 2-14)
	12	Surgery	Faucet	Nil
	13	Surgery	Faucet	Nil
	14	Surgery	Faucet	Nil
	15	Women	Shower	Nil
	16	Delivery	Shower	Nil
Hebron	1	Surgery	Faucet	$7.1*10^3$ CFU/L (L.p. 2-14)
	2	Surgery	Faucet	100 CFU/L (L.p. 2-14)
	3	Surgery	Faucet	Nil

	4	Surgery	Shower	$6.6*10^3$ CFU/L (L.p. 2-14)
	5	Surgery	Shower	$2*10^3$CFU/L (L.p. 2-14)
	6	ICU	Faucet	200 CFU/L (L.p. 2-14)
	7	ICU	Shower	300 CFU/L (L.p. 2-14)
	8	Pediatric	Faucet	$1.65*10^4$ CFU/L Biofilm:121CFU (L.p. 2-14)
	9	Pediatric	Faucet	$1.62*10^4$ CFU/L Biofilm:27cfu (L.p. 2-14)
	10	Pediatric	Shower	$1.15*10^4$ CFU/L (L.p. 2-14)
	11	Pediatric	Shower	$1.09*10^4$ CFU/L (L.p. 2-14)
	12	Pediatric	Shower	$1.16*0^4$ CFU/L (L.p. 2-14) Biofilm:10CFU
	13	Incubator	Faucet	800 CFU/L (L.p. 2-14)
	14	Delivery	Faucet	500 CFU/L (L.p. 2-14)
	15	Delivery	Faucet	Nil
	16	delivery	Shower	NIL
	17	Delivery	Shower	NIL
	18	Physical Medician Rehabilitation	Shower	NIL

Beit Jallah	1	Pediatric	Shower	$3*10^3$ CFU/L (L.p. 2-14)
	2	Pediatric	Shower	$2.1*10^3$ CFU/L (L.p. 2-14)
	3	Pediatric	Shower	$4.4*10^3$ CFU/L (L.p. 2-14)
	4	Pediatric	Shower	$6.5*10^3$ CFU/L (L.p. 2-14)
	5	Surgery	Shower	$1.2*10^3$ CFU/L Biofilm:2CFU (L.p. 2-14)
	6	Surgery	Shower	$3.6*10^3$ CFU/L Biofilm:1CFU (L.p. 2-14)
	7	Delivery	Shower	*$3.3*10^3$cfu/l* Biofilm:4CFU (L.p. 2-14)
	8	Delivery	Shower	$6.2*10^3$ CFU/L Biofilm:3CFU (L.p. 2-14)
Al-Wtani	1	Incubator	Shower	$1.4*10^3$ CFU/L (L.p. 2-14)
	2	Pediatric	Shower	500 CFU/L (L.p. 2-14)
	3	Pediatric	Shower	200 CFU/L (L.p. 2-14)
Rafidia	1	Incubator	Shower	Nil
	2	Delivery	Shower	200 CFU/L (L.p. 2-14)

	3	Burn	Shower	200 CFU/L (L.p. 2-14)
Jenin	1	delivery	Shower	$3.8*10^3$ CFU/L (L.p. 2-14)
	2	women	Shower	$2.85*10^4$ CFU/L (L.p. 2-14)
	3	incubator	Shower	$1.44*10^4$ CFU/L (L.p. 2-14)
	4	ICU	Shower	$2.15*10^4$ CFU/L (L.p. 2-14)
	5	surgery	Shower	$1.16*10^4$ CFU/L (L.p. 2-14)

Table A1.2: *P. aeruginosa* Results of West Bank Hospitals

Hospitals		Division	Source	Result
Ramallah	1	Pediatric	Shower	45CFU/200ml
	2	Pediatric	shower	10CFU/200ml
	3	Pediatric	shower	2CFU/200ml
	4	Pediatric	shower	1CFU /200ml
	5	Pediatric	faucet	4CFU /200ml
	6	Pediatric	faucet	18CFU/200ml
	7	Incubator	faucet	10CFU/200ml
	8	Surgery	shower	Nil
	9	Surgery	faucet	Nil
	10	Surgery	shower	Nil
	11	Surgery	faucet	Nil
	12	Surgery	faucet	Nil
	13	Surgery	faucet	Nil
	14	Surgery	faucet	Nil
	15	Surgery	faucet	Nil
	16	Surgery	faucet	Nil
	17	Surgery	faucet	Nil
	18	delivery	faucet	Nil
	19	delivery	faucet	Nil
	20	delivery	faucet	Nil
	21	delivery	shower	Nil
	22	women	faucet	Nil
	23	women	faucet	Nil
	24	women	shower	Nil
Alia	1	surgery	faucet	Nil
	2	surgery	faucet	Nil

	3	surgery	faucet	Nil
	4	surgery	faucet	Nil
	5	ICU	faucet	Nil
	6	Pediatric	faucet	Nil
	7	Pediatric	faucet	Nil
	8	Pediatric	faucet	Nil
	9	Pediatric	faucet	Nil
	10	Pediatric	faucet	Nil
	11	Pediatric	faucet	Nil
	12	incubator	faucet	Nil
	13	incubator	faucet	Nil
	14	delivery	faucet	Nil
	15	delivery	faucet	Nil
	16	delivery	faucet	Nil
	17	delivery	faucet	Nil
	18	delivery	faucet	Nil
Beit Jallah	1	Pediatric	Faucet	Nil
	2	Pediatric	Faucet	Nil
	3	Pediatric	Faucet	Nil
	4	Pediatric	Faucet	Nil
	5	Pediatric	Faucet	Nil
	6	Surgery	Faucet	Nil
	7	Surgery	Faucet	Nil
	8	Surgery	Faucet	Nil
	9	Delivery	Faucet	Nil
	10	Delivery	Faucet	Nil
	11	Delivery	Faucet	Nil
	12	Pediatric	Faucet	Nil
	13	Delivery	Faucet	Nil
Al-Wtani	1	Pediatric	Faucet	Nil
	2	Pediatric	Faucet	Nil

	3	Pediatric	Faucet	Nil
	4	Pediatric	Faucet	Nil
	5	Pediatric	Faucet	Nil
Rafidia	1	Incubator	Faucet	Nil
	2	Surgery	Faucet	Nil
	3	Delivery	Faucet	Nil
	4	Delivery	Faucet	Nil
	5	Delivery	Faucet	Nil
	6	Burns	Faucet	Nil
	7	Burns	Faucet	Nil
	8	Surgery	Faucet	Nil
	9	Surgery	Faucet	Nil
	10	Surgery	Faucet	Nil
	11	Surgery	Faucet	Nil
Jenin	1	Pediatric	Faucet	TNTC
	2	Pediatric	Faucet	TNTC
	3	Pediatric	Faucet	TNTC
	4	Delivery	Faucet	TNTC
	5	Delivery	Faucet	TNTC
	6	Delivery	Faucet	15CFU/200ml
	7	Women	Faucet	TNTC
	8	Women	Faucet	17CFU/200ml
	9	Incubator	Faucet	31CFU/200ml
	10	Surgery	Faucet	TNTC

Annex 2

Glossary

Aerosol: A suspension in a gaseous medium of solid particles, liquid particles or solid and liquid particles having negligible falling velocity.

The American Legion: is an organization of veterans of the United States armed forces who served in wartime. The American Legion is active in US politics. It's primary mission is to help American veterans and their families, as well as American children and young adults. American Legion was held in 1919.

Biofilm: A community of bacteria and other micro-organisms, embedded on any surface exposed to water and bacteria

Cooling tower: A device for cooling water that, in turn, is used for cooling other process fluids by use of a heat exchanger. The water is passed over the tower against an air stream. Water evaporates which causes the water to be cooled. It is then pumped back to the heat exchanger for further cooling of the process fluids before being recycled back to the cooling tower.

Cystic fibroses: One of the most common grave genetic (inherited) diseases, CF affects the exocrine glands and is characterized by the production of abnormal secretions, leading to mucous build-up.

This accumulation of mucus can impair the pancreas and, secondarily, the intestine. Mucous build-up in lungs tends progressively to impair respiration. Without treatment, CF results in death for 95% of affected children before age 5.

Dead-leg: A length of pipe leading (to a fitting) through which water only passes when the fitting is operated.

Distribution system: Pipework which distributes water from hot / cold / cooling water plant to one or more fittings/appliances

Legionella: A genus of aerobic bacteria, gram negative (of which there are over 48 species) that belongs to the family Legionellaceae. These are ubiquitous in the environment and found in a wide spectrum of natural and artificial collections of predominantly warm waters.it cause two forms of disease : Legionnaires' disease., and Pontiac fever disease.

Legionella pneumophila: The species of *Legionella* that most commonly causes Legionnaires' disease.

Legionnaire's disease is an acute respiratory infection caused by the bacterium *Legionella pneumophila*, which can cause a broad spectrum of disease from mild cough and fever to a serious pneumonia.

Legionellosis: Any illness caused by exposure to Legionellae.

Nosocomial: The term "nosocomial" comes from two Greek words: "nosus" meaning "disease" + "komeion" meaning "to take care of." Hence, "nosocomial" should apply to any disease contracted by a patient while under medical care.

A nosocomial infection is specifically one that was not present or incubating prior to the patient being admitted to the hospital, but occurred within 72 hours after admittance to the hospital.

Opportunistic pathogens: pathogen that exploits some break in the host defenses to initiate an infection.

Pneumonia: is an infection of one or both lungs which is usually caused by bacteria, viruses, or fungi. Some cases of pneumonia are contracted by breathing in small droplets that contain the organisms that can cause pneumonia. Once organisms enter the lungs, they usually settle in the air sacs of the lung where they rapidly grow in number. This area of the lung then becomes filled with fluid and pus as the body attempts to fight off the infection.

Pontiac fever: Milder self-limiting form of legionnaires'disease, primarily characterized by myalgia but no pneumonia

P*seudomonas aeruginosa*: is a gram-negative bacteria, aerobic rod belonging to the bacterial family pseudomonadaceae,. These bacteria are common inhabitants of soil and water. Is an opportunistic pathogen.

Sero-group: A sub-group of the main species determined by detection of specific antigens in or on the cell by the use of antibodies.

الملخّص

الكشف عن بكتيريا *Legionella* في مياه مستشفيات الضفة الغربية

تم عمل مسح لانتشار بكتيريا Legionella في أنظمةِ توزيع المياه الحارةِ في مستشفيات الضفة الغربية, و كذلك تم فحص بكتيريا Pseudomonas aeruginosa لوجود علاقة محتمله بينها و بين Legionella.

في هذه الدراسةِ تم جمع العينات من ست مستشفيات حكومية في الضفة الغربية و هي: مستشفى رام الله في رام الله و مستشفى عالية في الخليل، و مستشفى بيت جالا في بيت لحم , و المستشفى الوطني و مستشفى رفيديا في نابلس, و مستشفى جنين في جنين. كان مجموع العينات 134 عينة (53 عينة لتحليل Legionella و81 عينة لتحليل Pseudomonas aeruginosa).

تم عزل بكتيريا Legionella من نوع Legionella pneumophila sg (2-14) من 33 عينة بنسبة (62.3 %) من 53 عينةِ تم تحليلها. أما بكتيريا Pseudomonas aeruginosa فقد تم عزلها من 17 عينة بنسبة (21 %) من 81 عينة تم تحليلها.

لتَقييم أثر عملية التعقيم بالحرارةِ على بكتيريا Legionella pneumophila sg (2-14) , و بكتيريا Pseudomonas aeruginosa التي أجريت في مستشفى بيت جالا و مستشفى جنين, تم فحص عينات قبل و عملية التعقيم بالحرارةِ و بعدها, فكان الآتي:

في مستشفى بيت جالا تم رفع درجةِ حرارة الماءَ إلى 80 درجة مئوية لمدة 30 دقيقةِ، مع ابقاء جميع مخارج المياه مفتوحة طوال الفترة الزمنية و بهذا الماءِ الحار، فكانت النتيجة أن قلت نسبة التلوث من 100 % (قبل التعقيم بالحرارةِ) إلى 17 % (بعد التعقيم بالحرارةِ).

أما في مستشفى جنين فقد تم رفع درجةِ حرارة الماءَ إلى 70 درجة مئوية لمدة 30 دقيقةِ ، مع إبقاء جميع مخارج المياه مفتوحة طوال الفترة الزمنية و بهذا الماءِ الحار، , فكانت النتيجة انخفاض في تركيز Legionella و ليس قتلها و التخلص منها بالكامل.

أما بالنسبة لبكتيريا Pseudomonas aeruginosa عملية التعقيم بالحرارةِ على 70 درجة مئوية لمدة 30 دقيقةِ كانت فعالة في التخلص من البكتيريا و القضاء عليها. فنسبة التلوث قلت من 100 % (قبل التعقيم بالحرارةِ) إلى 0 % (بعد التعقيم بالحرارةِ).

في هذه الدراسةِ لوحظ بأن جميع أقسام الأطفال في مستشفيات الضفة الغربية ملوثة إما ببكتيريا pneumophila Legionella sg (2-14) أو Pseudomonas aeruginosa أو كلاهما, مما يشكل مصدر خطر على حياة الأطفال. بالإضافة إلى المغاسل الموجودة في غرفةِ الحاضناتِ حيث يتم حمام الأطفال الخُدّج ملوثة كذلك.