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Review Article

Legionella: Health Impacts, Exposure Evaluation, and Hazard Reduction

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ABSTRACT

Legionella pneumophila is an intracellular pathogen, omnipresent in the nature and seen as opportunistic. It is the main source of legionellosis that can take place in its nonpneumonic form (Pontiac fever) and acute pneumonic form (Legionnaires' disease). In the aquatic systems, *L. pneumophila* can conquer and remain alive intracellularly in different protozoans. The faculty to multiply inside biofilms gives more safeguard from natural stresses like disinfection. Human contagion by *L. pneumophila* happens following the inhalation or aspiration of aerosols carrying the pathogen. This work defines microbiologically *Legionella* bacteria and presents a brief history relating to their first discovery and following contagions, a short description relating to their metabolism and physiology, a discussion of their clinical characteristics and their subsistence in the nature and growth in a biofilm, and a general examination of numerous technologies employed for their removal. The spread of opportunistic pathogens (OPs) remains the most significant feature of microbial potable water quality besides the generation of disinfection by-products (DBPs). The (re)growth of OPs and the production of DBPs in urban engineered water systems both closely correlate with the injections or concentrations of disinfectant residuals. Nonetheless, OPs and DBPs respond to disinfectant residuals frequently oppositely. An elevated residual concentration efficiently suppresses the (re)growth of OPs while intensifies the production of DBPs. Oppositely, a low or "detectable" disinfectant residual level decreases the generation of DBPs but could not stop OPs from thriving. To guarantee that the overall or combined health risks of OPs and DBPs are minimum, OP (re)growth and DBP generation must be deeply revised while selecting a practical disinfectant residual dosage or level.

1. Introduction

Living in water, *Legionella* are bacteria that could provoke two kinds of illness in humans: Legionnaires' disease and Pontiac fever (PF) [1]. The first one is a grave respiratory disease that conducts to pneumonia [2]. The name *Legionnaires' disease* (LD) is from an outbreak of pneumonia that killed 29 people at an American Legion Convention in Philadelphia in 1976 [1-3]. As a rule, the average number of mentioned cases of LD is less than 100 per year in Canada, even if the real number of cases is suggested to be much bigger since several people with pneumonia may not be tested for infection with *Legionella* [1]. The second one is a milder illness provoking flu-like symptoms but not pneumonia and was primarily noticed in Pontiac (Michigan) in the early 1970s [1]. With PF, people usually recover during 2-5 days without treatment [1].
LD happens all around the globe, especially in summer and

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autumn, even if it is not a frequent illness [1,4]. This is may be explained by the fact that cases of Legionnaire's disease could be hard to detect as very few of the people exposed to the bacteria get infected (i.e., for 100 people exposed to *Legionella*, fewer than 5 of them will get Legionnaire's disease). However, for 100 people exposed to PF, 95 of them are possibly to become touched [1].

Further to pneumonia, symptoms (e.g., fever, cough, muscle pain and headache) start within 2-14 days of contagion and may continue for several months [1]. Most cases could be treated successfully with antibiotics, and efficacy of the treatment depends on age and on how quickly the person receives the right medical treatment.

Some people are at serious danger of developing the disease like persons over 40 years of age, people with chronic lung or kidney disease, people with diabetes, and people with weakened immune systems [1]. People in some occupations (like those who do maintenance work on large air-conditioning systems) could as well be at elevated danger of exposure to *Legionella* bacteria. Usually, more men than women contract LD and it is unusual for persons younger than 20 years of age to get LD [1].

Legionella bacteria are observed in natural water sources (e.g., lakes, rivers, ponds, and streams) mostly at levels that are too low to provoke disease in humans [1,5]. Cases of LD have happened in numerous different settings, comprising homes, commercial buildings, spas, cruise ships and health care facilities. LD cannot be diffused from one person to another. The number of bacteria determines the hazard for persons [6,7].

Some conditions, which may occur in buildings and homes raise the development of the bacteria, comprise stagnant water, warm water temperatures (20-50°C) and the occurrence of biofilm, scale and sediment [8]. Such conditions could be noticed in: cooling towers (like those employed with the air conditioning systems of large buildings); whirlpool bathtubs, hot tubs and public spas; plumbing systems (comprising water heaters, faucets and showers) either in the home or in larger buildings; and humidifiers [1,6,9].

When water polluted with larger amounts of *Legionella* is released into air in the form of droplets or mist, persons may be exposed to the bacteria by breathing in the polluted air [1].

Cooling towers could be an excellent habitat for *Legionella* to expand and such towers have the potential to release considerable quantities of water droplets to the air. For such cause, cooling towers are usually related to eruptions of LD. Droplets with elevated densities of *Legionella* could in that case come in a building if, for instance, the ventilation device intake is near the cooling tower. In many eruptions, nevertheless, *Legionella* bacteria from cooling towers seem to have persisted and diffused in the air over a distance of numerous kilometers. In such eruptions, cases were not related to a specific building or public space [1,10].

The danger of getting LD is usually very small [1]. At home level, the dangers may be minimized via correct maintenance of all mist-producing tools (e.g., shower heads, hot tubs, whirlpool bathtubs, and humidifiers). Such tools should be also periodically cleaned and disinfected following manufacturer guidance. Home water heater has to be maintained at a minimum of 60°C [11] to assist blocking the development of *Legionella*, even if temperature of water must no overpass 49°C to avoid the hazard of scalding. This is why mixing valves must be installed to regulate the tap water temperature [1].

Numerous accepted protocols for the protection of *Legionella* pneumonia use as a basis the type of intervention (to disinfect or not) on the degree of contagion observed (colony-forming unit, CFU/L). Nonetheless, if the degree of pollution by *Legionella* spp. of a water system varies in a short period of time, insufficient sampling may conduct to dissimilar decisions being made [5,12]. To decide if there are notable disparities in the bacterial count of *Legionell*a spp., water samples must be taken at several periods from the same sites. The Italian Guidelines suggest disinfection only for a *Legionella* count >10,000 CFU/L in hospitals without documented cases of disease.

This work firstly defines microbiologically *Legionella* bacteria and presents a brief history relating to their first discovery and following contagions. To understand *Legionella*'s behavior, a short description relating to their metabolism and physiology is given. As humankind is concerned by *Legionella* contagion, their clinical characteristics are discussed besides their subsistence in the nature and growth in a biofilm. A general examination concerning different techniques used for controlling such bacteria is finally presented.

2. Microbiological viewpoint

Legionellae are Gram-negative and non-spore-forming bacteria belonging to the bacterial class, Gammaproteobacteria [2,9,13]. Such bacteria are short (about 0.3-0.9 μm wide and 1-3 μm long) rod-shaped cells; further, they usually defined as coccobacillary [14]. The rods may be nonuniform in shape, with non-parallel sides; further, in older cultures, long filamentous forms of *Legionella* have been mentioned. The typical species of the genus is *Legionella pneumophila* that is the cause of

legionellosis [2].

Excepting *Legionella oakridgensis*, *Legionella londinensis* and *Legionella nautarum*, all *Legionella* are motile in wet mounts and inside contaminated cells. Motility is promoted by one or more polar or subpolar unsheathed flagella [2]. In enriched media, pili and fimbriae have been detected. *Legionella* are strict aerobes and because of their fastidious nature will not grow on traditional bacteriological media as they need an enriched medium supplemented with L-cysteine and ferric salts. Optimal growth temperature for *Legionella* is 35°C. Legionella are catalase-positive and are incapable to reduce nitrate. Such bacteria as well do not use carbohydrates by either oxidation or fermentation.

Clinically, *Legionella* provokes legionellosis. Such word represents a group of diseases that change from mild febrile illness (i.e., PF), to a severe pneumonia known as LD. Such name, as mentioned above, arises from a pneumonia eruption in 1976 provoked by *L. pneumophila* that touched members of the Pennsylvania American Legion during a meeting at the Bellevue-Stratford Hotel, Philadelphia. Vulnerability to LD depends on age, underlying illness, immunosuppression and different hazard factors like smoking [2]. As the milder pneumonia, PF is called following the town, Pontiac, where employees and visitors of the health department suffered an acute respiratory disease [2].

The *Legionella* genus includes 50 species that are subdivided into 70 distinct serogroups [15]. *L. pneumophila* (mainly serogroup 1) is the most usual and well-known pathogen inside the genus. Such species remains the causative agent of more than 70% of legionellosis. In non-*L. pneumophila* contaminations, the causes have mostly been *Legionella micdadei* (60%), *Legionella bozemanii* (15%), *Legionella dumoffii* (10%), *Legionella longbeachae* (5%) and different species (10%) [2,4].

Both LD and PF happen because of aspiration of polluted aerosols [2,16]. As mentioned above, aerolization may arise from air-conditioning systems, whirlpools, fountains and even dental devices [15,17].

In water, *Legionella* is able to remain alive intracellularly inside protozoan parasites and the kept environment given by the protozoan envelope diminishes its vulnerability to disinfection and different damaging situations [2,18].

3. Natural history, metabolism and physiology, and clinical characteristics

3.1. Natural history

In 1943, the earliest *Legionella* were isolated from guinea pigs [19]. Ten years later, a bacterium was isolated from 'free living' protozoa, even if this was not classified as a species of *Legionella* until 1996 [20]. It was not until 1979 that the genus *Legionella* and the species *L. pneumophila* were in fact detected [2]. This happened after the famous eruption of contagion in 1976, as mentioned previously, during which, 221 attendees fell ill with an apparent pneumonia and of these, 34 died [21]. After this incident, *Legionella* was recognized as the source of several different large eruptions of identical diseases. Following serological investigations from former eruptions of an identical type permitted the retrospective identification of *L. pneumophila*. Moreover, *Legionella* is accountable for a comparatively mild, self-limiting, influenza-like illness, named *Pontiac fever* (PF) [22]. PF appears in otherwise healthy persons as pleuritic pain in the absence of pneumonic or multisystem manifestations. The condition carries a short incubation period, a high attack rate, but a very low mortality ratio [2].

Legionella is the one and only genus inside the family Legionellaceae [2,23]. Such family is constituted of numerous species and serogroups (as listed in Table 1), and according 16S rRNA analysis, has been revealed to be a member of the gamma-2 subgroup of the class Proteobacteria. A near genealogical to Legionella is the bacterium *Coxiella burnettii*, which depicts identical intracellular survival and utilizes common genes to provoke contagion [24].

Table 1. Clinical differentiation of legionellosis (i.e., Legionnaires' disease (LD) and Pontiac fever (PF)) [2].

	Legionnaires' Disease (LD)	**Pontiac Fever (PF)**
Incubation time (days)	2-10	1-2
Attack rate (%)	1-5	95
Case-fatality ratio (%)	0-20	0
Called for	Philadelphia outbreak (in 1976)	Pontiac outbreak (in 1968)
Clinical syndrome	Pneumonia	Non-pneumonic
Symptoms common to both	Fever, cough, headache, confusion, chest pains, nausea, malaise, diarrhoea and vomiting	

Symptoms unique to each	Dyspnoea, haemoptysis, upper respiratory tract infection, sputum production, abdominal pain, pleuritic pain
Other organs affected	Central nervous system, gastrointestinal tract
Incidence rates	Change but range from 1 to 30 % of all pneumonias

As aforesaid, there are over 50 species of Legionella comprising 70 serogroups [25]. There are 15 serogroups of *L. pneumophila* and two in *L. longbeachae*, *Legionella hackeliae*, *Legionella sainthelensi*, *Legionella spiritensis*, *Legionella erythra*, *Legionella quinlivanii*, *L. bozemanii* and *Legionella feeleii* with only a single serotype found in the other group members [2].

3.2. Metabolism and physiology

Legionellae are obligate aerobes and nutritionally fastidious [2]. The bacteria are catalase positive (weakly) and oxidase variable. Nitrate is not reduced and urea is not hydrolyzed. Most species of *Legionella* generate a β-lactamase and as well liquefy gelatin, with the remarkable special case of *L. micdadei*. Further, *L. pnuemophila* (except serogroups 4 and 15) all hydrolyze hippurate; and, in fact, this is a helpful differentiating test since the majority of other *Legionella* species are negative for such reaction. Legionellae employ amino acids for energy rather than carbohydrates, and the latter are thus neither oxidized nor fermented. Because amino acids work as the carbon source for legionellae, essential amino acids needed by all isolates comprise, arginine, cysteine, methionine, serine, threonine and valine; however, other strains as request isoleucine, leucine, phenylalanine and tyrosine for growth [26].

Legionella are superoxide dismutase-positive, weakly peroxidase-positive and have cytochromes *a–d* [2]. Legionellae have an absolute need for iron, with surplus quantities generally requested to attain optimal growth and for the efficient operation of bacterial enzymes like ferredoxins and cytochromes. Iron is as well implicated in the processes of electron transport, regulation of gene expression and oxygen metabolism.

Intracellular replication of the organism happens during illness, and iron is crucial for such process; thus, iron chelators (e.g., apolactoferrin) impede intracellular growth of the organism. Ferritin, lactoferrin and transferrin work as the major sources of iron for host cells and it has been suggested that ferritin may work to recycle iron within the iron pool available to the organism within macrophages following degradation and iron release in the lysosomes; nevertheless, the accurate route by which iron is taken up by the organism remains unclear [2].

In terms of cellular fatty acids, *Legionella* hold high levels of branched-chain cellular fatty acids [2]. *Legionella* as well hold ubiquinones (coenzymes implicated in electron transport), which possess 9-14 isoprene units in the side chains. Founded on ubiquinone content, subdivision into distinct groups have been delineated.

3.3. Clinical characteristics

Even if 15 serotypes of *L. pneumophila* exist, roughly 70% of all culture or urine antigen confirmed cases are provoked by *L. pneumophila* serogroup 1 [2]. About 50% of all *Legionella* species have been announced to be related to human illness. The illness is common in public health professionals and persons implied in building water system maintenance.

As aforesaid, legionellosis usually appears as two clear disease entities (i.e., LD that is a severe multisystem disease implying pneumonia, and also PF that is a mild non-pneumonic self-limited flu-like illness with a high attack rate) [2]. Even if LD is identified as an acute pneumonia, with a high fatality rate (~ 10%), it is as well recognized to touch the nervous system, gastrointestinal system and urinary system. Such extra-pulmonary syndrome takes place when *Legionella* spreads from the lungs to other body sites [27]. It is not easy to clinically differentiate patients with LD and those with other types of pneumonia [2].

The incubation time for LD takes usually 2-14 days, and the infection may endure weeks to months [2]. First symptoms comprise fever, a non-productive cough, headache, generalized weakness, myalgias, rigors, diarrhoea, delirium and dyspnoea. Bloody or purulent sputum could be formed late in the illness in company with advancing respiratory complications. In PF, symptoms implicate fever, chills, myalgia and headache. The incubation period for PF takes 5-66 hours and symptoms persist for 2-7 days.

4. Subsistence in the nature

Legionellae have been observed in lakes and rivers [2], drinking hot water [28], cooling towers [29], whirlpools and ground water [30]. Devos et al. [31] observed Legionellae in around 40% of freshwater environments by culture and up to 80% utilizing polymerase chain reaction (PCR), and found that 56% of 46 aquatic samples (shower, industrial, natural and tap water) were positive for *Legionella* species by cultivation and PCR [31]. Another study [32] noticed that *Legionella* was existing at more

elevated levels than other bacteria in surface or ground waters, and the plurality of isolates observed were *L. oakridgensis* and *L. pneumophila* [2]. Mansi et al. [13] focused on the usability of a quantitative PCR (qPCR) technique integrated with ethidium monoazide (EMA) to the quantification of *Legionella* spp. in samples collected from swimming pools, water recirculation systems and hot water systems in two fitness clubs. Such molecular method (EMA–qPCR) lets the amplification of target deoxyribonucleic acid (DNA) from culturable and viable cells, however averts the amplification of DNA from non-viable cells. They established that EMA–qPCR permits to distinguish the non-viable cells from those viable and that it is especially indicated for controlling the performance of thermal treatments for the *Legionella* contagion control in water systems, as well furnishing data concerning the occurrence of Viable But Non-Culturable (VBNC) cells [13].

Bacterial regrowth could indeed happen via reactivation from a VBNC state, repair of photo-induced DNA damage, and reproduction of bacteria surviving disinfection [33,34]. Numerous investigations have underestimated the level of actual regrowth due to the usage of simple experimental designs and plate count methods, which cannot quantify actual abundance of viable bacteria (Fig. 1) [33]. More attention should be given to the influences of numerous elements on bacterial regrowth in realistic circumstances in regrowth experiments and consider multiplex detection methods that integrate culture-based and culture-independent approaches [35]. A detailed comprehension of the routes implied in bacterial regrowth after disinfection remains crucial for safeguarding public health and aquatic environments.

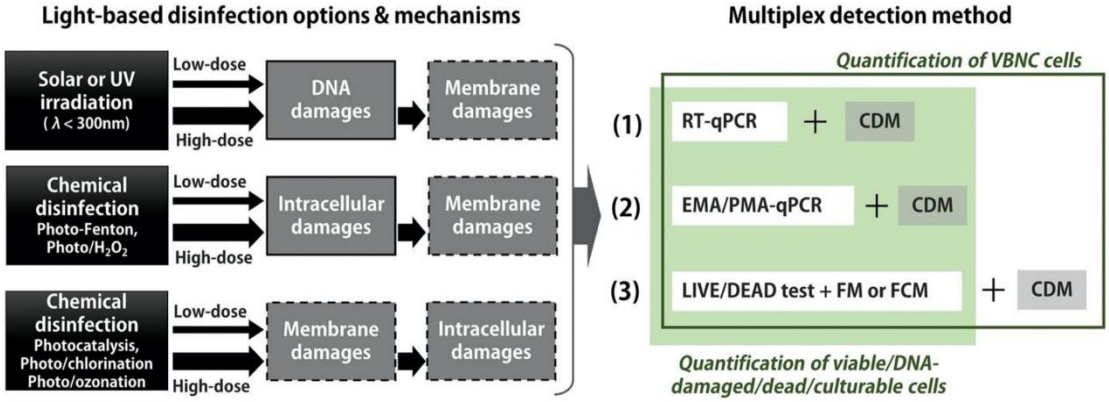

Fig. 1. Suggested multiplex detection procedure for measuring bacterial regrowth following different light-based disinfection techniques. qPCR, quantitative polymerase chain reaction; RT-qPCR, reverse transcription qPCR; EMA, ethidium monoazide; PMA, propidium monoazide; FM, fluorescence microscopy; FCM, flow cytometry; CDM, culture-dependent method (i.e., plate count method); VBNC, viable but non-culturable [33].

Additional Gram-negative bacteria usually linked with biofilm were detected in samples taken from swimming pools and balance tanks, proposing that as well the occurrence of biofilm has to be controlled for a more global monitoring of water pollution [2].

Concentrations of *Legionella* in water are inclined to be more elevated during summer even if numbers do not inevitably correspond with those of coliforms, *Escherichia coli*, intestinal enterococci or *Clostridium perfringens* [36]. Greater counts of *Legionella* are observed in man-made water supplies comprising hot water systems especially in hospitals, hotels and cooling towers [2,37]. Long-term subsistence of *Legionella* in tap water has as well been confirmed [38].

Even if persons are easily touched by *Legionella*, they remain inconvenient hosts, providing no ecological benefit to the organism [2]. Delivery of the agent to the respiratory tract in the form of water droplets (5-15 mm in diameter) works as the principal channel for illness diffusion. *L. longbeachae* remains a recurrent source of legionellosis in gardeners in Australia and USA and this has been related to its elevated spread in potting soil [2].

Legionella are not usually seen to be thermophilic, regardless of being isolated from waters at temperatures as high as 60°C [2]. *L. pneumophila* develops at temperatures between 25°C and 42°C (the optimal growth temperature is around 35°C). The presence of *Legionella* in water reservoirs does not inevitably conduct to eruptions of illness, nor to augmented frequency of occasional contagions [39]. Nevertheless, aerosols carrying the organism could form a main hazard agent for nosocomial contagions and contaminations in the immunocompromised [16]. It is the aerosol route of transport that motivate the obligation to both detect and eradicate the organism from colonized water reservoirs (mostly in hospitals). Most cases of legionellosis could be

related to human aquatic environments in which the water temperature is higher than the ambient temperature. In fact, without man-made aquatic environments, legionellosis would be a scarce illness, since natural freshwater environments are not involved with contagion.

Warm water temperature and the occurrence of nutritional elements permit legionellae to develop in water [2]. The existing concentrations of nutrients detected in freshwater are, in fact, seldom abundant for development of legionellae. Legionellae could remain alive in aquatic and soil environments in association with free living protozoa and development of legionellae in the absence of protozoa has only been noticed in laboratory media.

Legionellae multiply intracellularly in amoebae and ciliates belonging to the genera *Hartmanella*, *Acanthamoeba*, *Naegleria*, *Echinamoeba*, *Tetrahymena* and *Cyclidium* [40-42], particularly while water temperatures are high [2,43]. Intracellular replication inside protozoa could help this otherwise nutritionally fastidious organism via favoring subsistence in the poor aquatic environment [44]. As an auxiliary result of intraprotozoal subsistence, virulence in human contagion [45] could be increased in that protection is afforded to the organism within protozoal cysts, which display augmented resistance to drying and to water treatment techniques (e.g., chlorination and heating) [46-48]. Further, microbial counts could be augmented in aerosols carrying such protozoa. By such manner, transport of the pathogen in an amplified, protected and pre-packaged aerosolized form to the lungs of vulnerable persons eases contagion. The phenomena that permit the subsistence and replication of the organism inside protozoa could be reflected in human alveolar macrophages [40,49]. It is likely that such routes implied in the molecular recognition between legionellae and protozoa or macrophages are identical; in both cases, uptake, intracellular replication and dissemination follow (Fig. 2) [50].

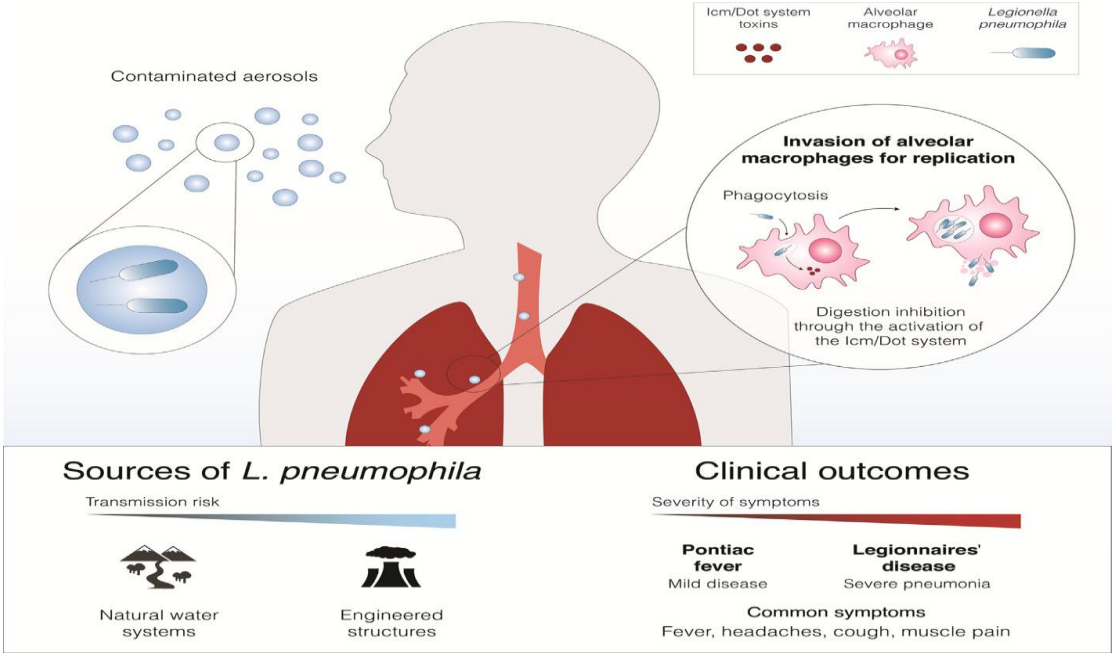

Fig. 2. *Legionella pneumophila* (*L. pneumophila*) is an intracellular pathogen, omnipresent in the nature and seen as opportunistic [50].

4.1. Subsistence in water and epidemiology

Investigation into drinking water reservoirs has been incapable to determine the levels of legionellae requested to initiate illness [2]. Nevertheless, as a rule, concentrations over 10^4-10^5 CFU/L of *Legionella* are counted a possible danger to human health and are linked with legionellosis [2]. While *Legionella* has been isolated in domestic households, it should noticed that regardless of their occurrence, there are frequently no visible marks of related illness. This may, nonetheless, be a significant health regard, especially if counts move to elevated levels inside biofilms and are then sloughed off at the consumer's tap.

As aforesaid formerly, investigation has established that *L. pneumophilia* is capable to develop inside the protozoa frequently discovered in water reservoirs [2,48]. This certainly provides a preservative habitat to *Legionella* versus unfavorable natural circumstances, and when *Legionella* come out from such host cells, they seem to be much more resistant to the action of biocides than bacteria grown under natural laboratory circumstances. This has consequences on the procedures adopted to control and eliminate *Legionella* in drinking water reservoirs.

If circumstances are adverse for protozoa (impoverished

nutrient availability or dry circumstances), the protozoa have tendency to develop a hard and impervious outer protective shell (named a *cyst*) [2]. Such cyst provides an even greater protective environment versus drying, extremes of temperature and biocide treatment. Since the bacteria are inside cysts, air currents may easily disperse them. When circumstances are appropriate to development of protozoa, the cysts open and the protozoa, jointly with *Legionella* are liberated. Such means of protected survival, under very severe circumstances, underlines the crucial requirement for good hygiene customs.

Water temperature and as well plumbing material are significant considerations for the development of both the protozoal host and *Legionella* itself [2]. It is well-known that *Legionella* and protozoa expand best at between 30°C and 40°C. Employing suggested temperatures outside such span in hot and cold-water reservoirs has assisted to restrict the epidemiological diffusion of *Legionella*. Water must be supplied and distributed below 20°C because *Legionella* are helpless to develop at such temperature. Inside hot water devices *Legionella* are only fit to persist for a few minutes above 50°C unless, as discussed previously, it is protected inside a cyst.

For control of *Legionella*, excellent management application is requested concerning systematic examination, maintenance and cleaning of water devices jointly with the integration of biocides [2].

LD possesses a planetary occurrence and accounts for 1-4% of all cases of pneumonia, even if rates as elevated as 30% have been noticed [51]. Nonetheless, the occurrence of PF remains undisclosed [2]. Ameliorations in laboratory-founded examinations have conducted to improved detection of LD; nevertheless, whether this is due to higher consciousness of the circumstance or to a real augmentation in the happening of the illness is not obvious. Asymptomatic seroconversions are not frequent and result from rare subclinical infections and low-grade cases of PF.

Human-to-human diffusion of *Legionella* is not supposed to take place. Illness not only happens in thrilling eruptions related to hospitals, hotels and large building complexes, but could as well be sporadic, nosocomial and community-acquired. The contagious dose stays unrevealed [2].

Acquisition of the illness could be related to the inhalation of polluted aerosolized water [2,52,53]. The origins of which comprise air-conditioning condensers, cooling tower effluent, humidifiers, nebulizers, potable and hot water supplies, domestic and hospital showerheads, whirlpool spas, decorative fountains and vegetable misting machines [54-56].

Aerosolization stays a main hazard agent; nonetheless, colonization of the organism inside water systems solo does not automatically conduct to eruptions [2]. The huge majority of *Legionella* illness is provoked by *L. pneumophila*, serotype 1 accounting for 50% of contagions, pursued by serotype 6 (10%), other *L. pneumophila* serotypes account for 20% and *L. micdadei* accounts for 5%. Different species of *Legionella* are obvious in the nature, even if are so seldom involved in illness. Such comprise *L. bozemanii*, *L. longbeachea*, *Legionella jordanis*, *Legionella wadsworthii*, *Legionella birminghamensis*, *Legionella cincinnatiensis*, *L. oakridgensis* and *Legionella tucsonensis* [9,57].

LD depicts a more elevated summer occurrence, probably because of augmented contact with the natural habitat of the organism [2].

4.2. Development in a biofilm

Legionellae may be revealed utilizing swab samples from biofilms inside water reservoirs, proposing a subsistence procedure for the organism in biofilms [2,58,59]. Therefore, the subsistence and multiplication of legionellae in biofilms are actually believable and would propose ameliorations in control pathways (Fig. 3). *Legionella* species have been detected in biofilms in domestic water systems and as well in 'floating biofilms' that have been emphasized as a specific ecological niche for the subsistence and multiplication of *Legionella*. Declerck et al. [60] detected *Legionella* in 100% and 81% of biofilms from anthropogenic and natural aquatic systems, respectively. Further, *Naegleria* species and *Acanthamoeba* species were existing in elevated parts in such biofilms. Such protozoa harbor *Legionella* [61]. Declerck et al. [62] employed *in vitro* models to illustrate proof that *Acanthamoeba castellanii* occurrence conducts to elevated levels of biofilm-associated *L. pneumophila*. In their tests, a reactor container was preconditioned with *Aeromonas hydrophila*, *E. coli*, *Flavobacterium* breve and *Pseudomonas aeruginosa*, forming a mixed species biofilm. When *L. pneumophila* was introduced, it was found in the biofilm following only 2 hours, showing that *Legionella* was capable at quickly colonizing the biofilm. The introduction of *A. castellanii* to the biofilms augmented counts of *Legionella* [62].

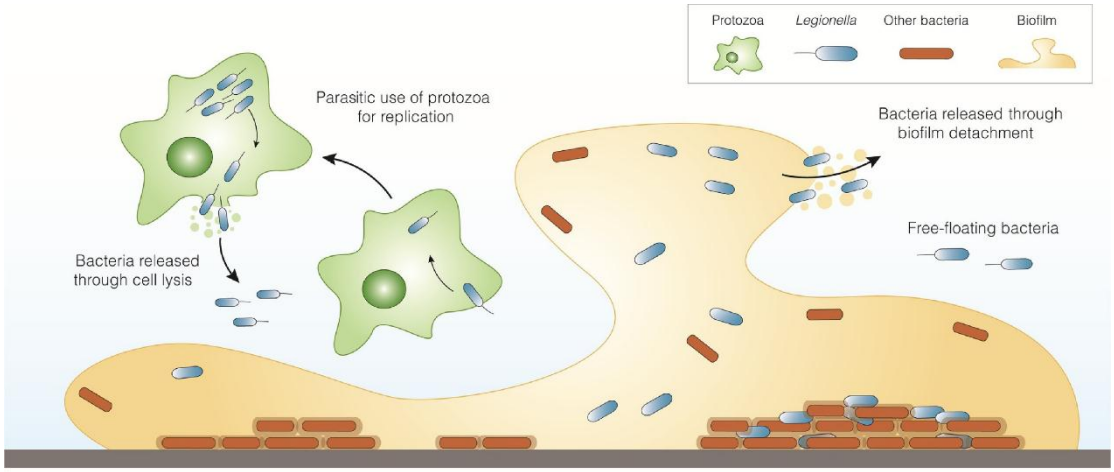

Fig. 3. The potential to grow inside biofilms allows extra protection from environmental stresses like disinfection [50].

5. Disinfection performance and mechanisms for Legionella control

Kim et al. [63] discussed the effectiveness of killing agents versus *Legionella* [2]. Disinfection decreases concentrations of *Legionella* in water reservoirs, even if long-term reduction from water has not been easy even in chlorinated devices [64]. Monochloramine is rated more performant than chlorine in eliminating *Legionella* [65,66], and chlorine dioxide has been illustrated to be efficient [67,68], especially when the organisms are linked with biofilms [58,69].

As mentioned above, *Legionella* are related with 'free-living' protozoa; further, beside with biofilm growth, such properties seem to be essential to the organism surviving disinfection activity [2,18,64]. As an illustration, hypochlorite at 256 mg/L minimizes *L. pneumophila* to unnoticeable concentrations; however, when the bacteria are inside *Acanthamoeba polyphaga*, resistance to 1024 mg/L hypochlorite is clear. Furthermore, resuscitation of harmed *Legionella* takes place when co-cultured with *A. polyphaga* [64]. Considerably more elevated resistance to chlorine and monochloramine remains as well observed when *L. pneumophila* are linked with the protozoan *Hartmannella vermiformis* [70]. Casini et al. [71] established the emergence of VBNC *Legionella* throughout a long period of continuous monochloramine treatment of a hospital water network, underlying the significance of maintaining an enough and unbroken monochloramine injection to make sure the monitoring of *Legionella* colonization in hospital water reservoirs (Fig. 4). Disinfection by ultraviolet (UV) irradiation could constitute an efficient technique to avert nosocomial LD [47,72-74]. Encouraging findings reached show the potential implementation of power ultrasound in controlling both *Legionella* and *Acanthamoeba* levels in anthropogenic water systems [75]. An ultrasonic treatment device, employing a TiO_2 photocatalyst, was utilized to kill *L. pneumophila* [76]. The pathway of cell killing was followed via studying the influences of $^{\bullet}OH$ radical scavengers (e.g., ascorbic acid, histidine and glutathione). The disinfection efficiency was decreased in samples that carried such radical scavengers, therefore illustrating the significance of $^{\bullet}OH$ radicals [76]. Photocatalytic oxidation (PCO) was established to be performant in demobilizing *L. pneumophila* [77]. The demobilization pathways of PCO were examined. PCO was observed to disintegrate the cells eventually; prior such disintegration, there was lipid peroxidation of outer and cytoplasmic membrane provoking holes generation and conducting to the entry of $^{\bullet}OH$ into the cells to oxidize the intracellular components. Fatty acid profile analysis discovered that the quantity of saturated, 16-carbon branched-chain fatty acid, which is predominant in *Legionella*, diminished in the surviving populations from PCO. A relationship between the amount of this fatty acid and the PCO sensitivity of the tested strains was also observed. Mineralization of cells by PCO was confirmed by total organic carbon analysis [77]. Polo-López et al. [57] demobilized *L. jordanis* in water using solar photocatalytic (TiO_2 and TiO_2/H_2O_2) and solar photochemical (solar/H_2O_2 and solar disinfection) techniques in distilled water under natural sunlight. Faster bacterial demobilization was reached employing 500-10 mg/L of TiO_2 and H_2O_2, respectively. Performance order of demobilization was: TiO_2/H_2O_2/solar (5 min) > TiO_2/solar (15 min) ≈H_2O_2/solar (15 min) > Solar only disinfection (90 min) [57]. They confirmed the well adopted route of TiO_2-photocatalysis via oxidative attacks of the external cell membrane. Further, using their experimental proofs, they reinforced the suggested route for H_2O_2/solar founded on internal photochemical reactions, since no deterioration of cell membrane was observed [57].

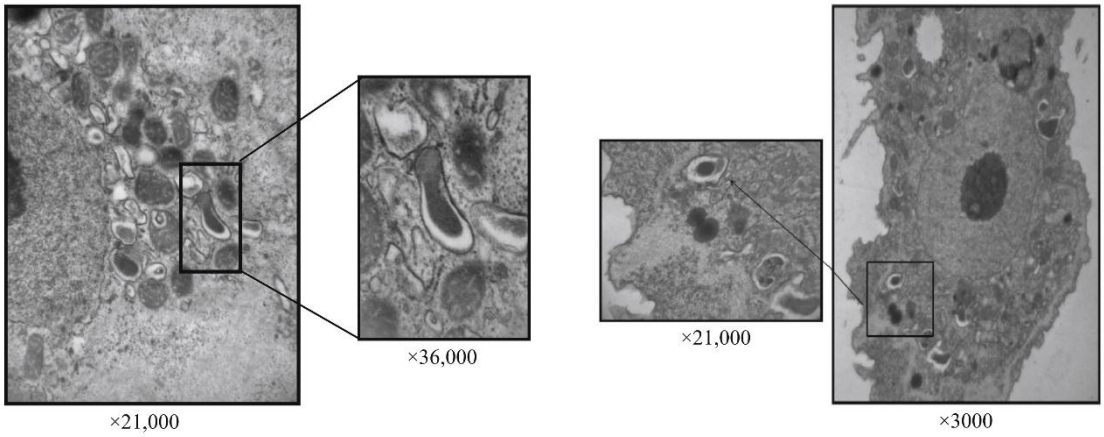

Fig. 4. Findings of resuscitation trials of viable but non-culturable (VBNC) *Legionella* cells in *Acanthamoeba polyphaga* (transmission electron microscope: ×21,000, ×36,000) [71].

Hungering *Legionella* of vital nutrients augments their resistance to chlorine and heat [78,79]; further, interaction with different microorganisms inside biofilms as well diminishes vulnerability [2]. Many *in vitro* investigations focused on elements influencing resistance of *Legionella* [80-82]. For that reason, removing and reducing biofilms and protozoa are seen crucial in attempting to diminish *Legionella* concentrations. Employing two materials frequently utilized for the delivery of drinking water as a substratum, copper and stainless steel, the growth of *L. pneumophila* biofilms and their response to chlorination was controlled during a three-day and a three-month period, respectively [83]. *In vitro* trials utilizing broth and sterile tap water as culture media depicted that the bacterium was apt of remaining in low numbers for 28 days in the existence of chlorine. Subsequently, biofilms were developed for three days, one month and two months, respectively, on stainless steel and copper sections, which are largely employed for the conveyance of drinking water. Immediately after exposure to 50 mg/L chlorine for 1 h, the biofilms yielded no recoverable colonies, but colonies did reappear in low numbers over the following days. Despite chlorination at 50 mg/L for 1 h, both one- and two-month-old *L. pneumophila* biofilms were able to survive this treatment and to continue to grow, ultimately exceeding 1 × 10^6 CFU per disc [83]. Steinert et al. [23] focused on the elements implicated in the presence of Legionellaceae in a hospital water system and examined the recontamination by *L. pneumophila* following a thermal disinfection application. Three months following the heat treatment (70°C), the regrowth (Fig. 5) of the two prevalent *Legionella* strains *(L. pneumophila* serogroup 1 [Oxford-like] and *L. pneumophila* serogroup 2) attained the initial degree of cell numbers. They proved that the serogroup 1 strain depicted a higher tolerance to 60°C than the serogroup 2 strain, which could account for the order of reappearance of the strains after the heat treatment. Potential host amoebae, comprising *Acanthamoeba* spp. and *Vahlkampfia* spp., which are known to play a critical role in the amplification process of *Legionella*, were isolated from the plumbing system. They also established both *Legionella* strains for an identical rate of multiplication in *A. castellanii*. In competitive coinfections, nonetheless, the serogroup 1 strain achieved a higher rate of multiplication if compared with the serogroup 2 strain [23]. Several researchers (e.g., Cervero-Aragó et al. [42] and He et al. [84]) furnished direct proof that viable and demobilized amoeba spores have the potential to keep safe their hitchhiking bacteria from disinfection treatment that is pivotal for future decision-making regarding the injection for adequate bacterial disinfection in potable water setups.

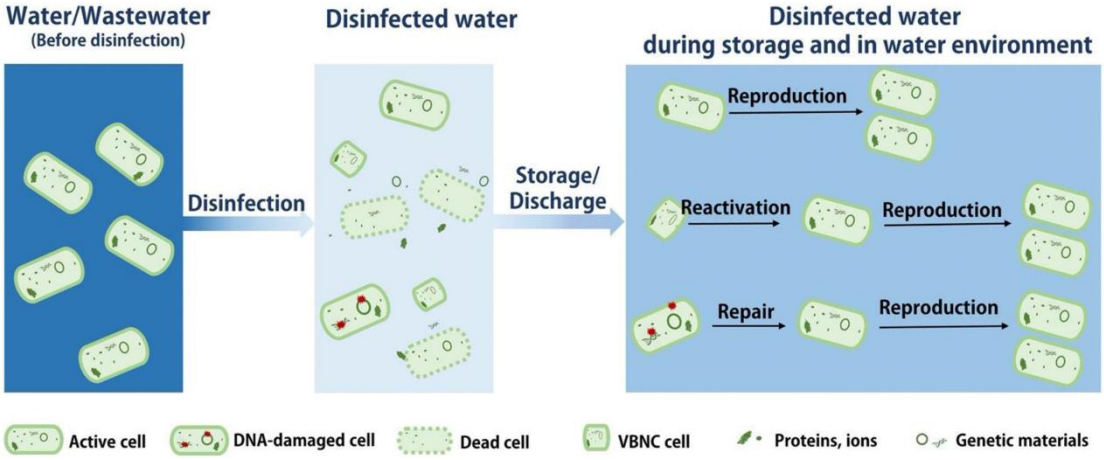

Fig. 5. Bacterial regrowth pathways: reactivation, repair, and reproduction. VBNC: viable but non-culturable; genetic materials include chromosome DNA and plasmid DNA [33].

Control procedures using elevated temperatures (50°C-65°C) remain performant in suppressing *Legionella*, at the same level with employing silver integrated with copper ions [85-87]. Thermal disinfection stays most efficient at >60°C and oxidizing chemicals remain frequently more performant than non-oxidizing ones [2,88]. Papagianeli et al. [89] suggested a predictive mathematical model depicting the influence of temperature on the demobilization of *L. pneumophila* in water, which was controlled under isothermal conditions (51-61°C). The findings illustrated that the model could successfully predict thermal demobilization of the pathogen at dynamic temperature circumstances and efficiently translate water temperature profiles to cell number decrease. Using such model besides efficient temperature control can furnish the foundation of a combined preventive procedure for the performant monitoring of *L. pneumophila* in plumbing devices [89]. Employing a reactor fed with tap water carrying 0.15 mg/L chlorine, Lehtola et al. [90] observed that *L. pneumophila* has the potential to remain alive in potable water-related biofilms for longer times than *E. coli*. Most importantly, a *L. pneumophila* strain was discovered to have persevered for 15 years in the water system of a hospital in Northern Italy in defiance of employing disinfection [91]. Cervero-Aragó et al. [11] affirmed that a prolonged thermal regime >60°C at the central parts of warm water systems is not only efficient towards culturable *L. pneumophila* but in the long run even against VBNC cells. Rasheduzzaman et al. 2020 [92] concluded that more investigation or more detailed reporting of existing datasets is needed to evaluate if *Legionella* development could be restricted below particular concentration targets at different temperatures.

Even if ozone could be an efficient killing agent, its usage for *Legionella* monitoring in a hospital water system was not effective [86]. Copper-silver ionization is greatly performant, with a condition that an adequate level of the ions is reached, even if this might not be easy due to restrictions dictated by national water regulations [86,93]. Nonetheless, an eruption of *Legionella* pneumonia took place at a university hospital employing copper-silver ionization for drinking water disinfection, especially following structural disruptions [94,95]. Keeping the water temperature above 50°C confirmed to be the most efficient monitoring procedure in some hospitals [86]. Amara et al. [96] affirmed that despite the fact that numerous monitoring processes may be found for disinfecting water (e.g., biocide, UV light sterilization, copper-silver ionization, ozonation, etc.), there is only thermal treatment that has the potential to reach total removal of *Legionella*, which is eliminated almost instantly at 70°C (Fig. 6). They analyzed *Legionella* disinfection (Fig. 7 [43]) using a solar concentrator merged with a heat recovery setup that decreases the heat demand [96]. Consecutive ozone□chlorine disinfection would be a proper mohair for killing *Legionella* spp. Cao et al. [97] juxtaposed the demobilization performance of *E. coli* via single ozone, single chlorine, and consecutive ozone□chlorine disinfection methods. A single ozone or chlorine process can only reach a log removal rate of up to 5 log, while the consecutive ozone□chlorine disinfection can totally demobilize pathogens (7.3 log). For consecutive ozone□chlorine disinfection, the efficacy of chlorination was enhanced by 2.4%-18.5%. The synergistic impact mainly imputed to the removal of chlorine consuming substances by ozone [97]. Li et al. [98] investigated *Legionella* demobilization employing O_3 in wastewater utilizing kinetic analysis and modeling. They depicted that the relationship between the O_3 level, germ level, and chemical oxygen demand (COD) could be employed to predict changes in germ and COD levels. The O_3 reaction with COD and demobilization of *Legionella* took place at the same time; however, the reaction with COD possibly took place at a higher rate than the demobilization, since

COD is more simply oxidized by O_3 than *Legionella*. Higher initial COD levels led to a lower demobilization rate [98].

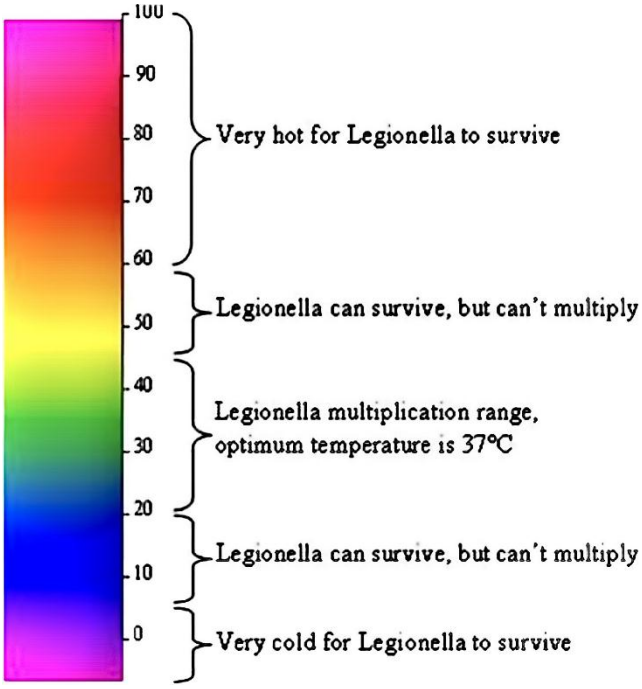

Fig. 6. *Legionella* occurrence under temperature conditions [96].

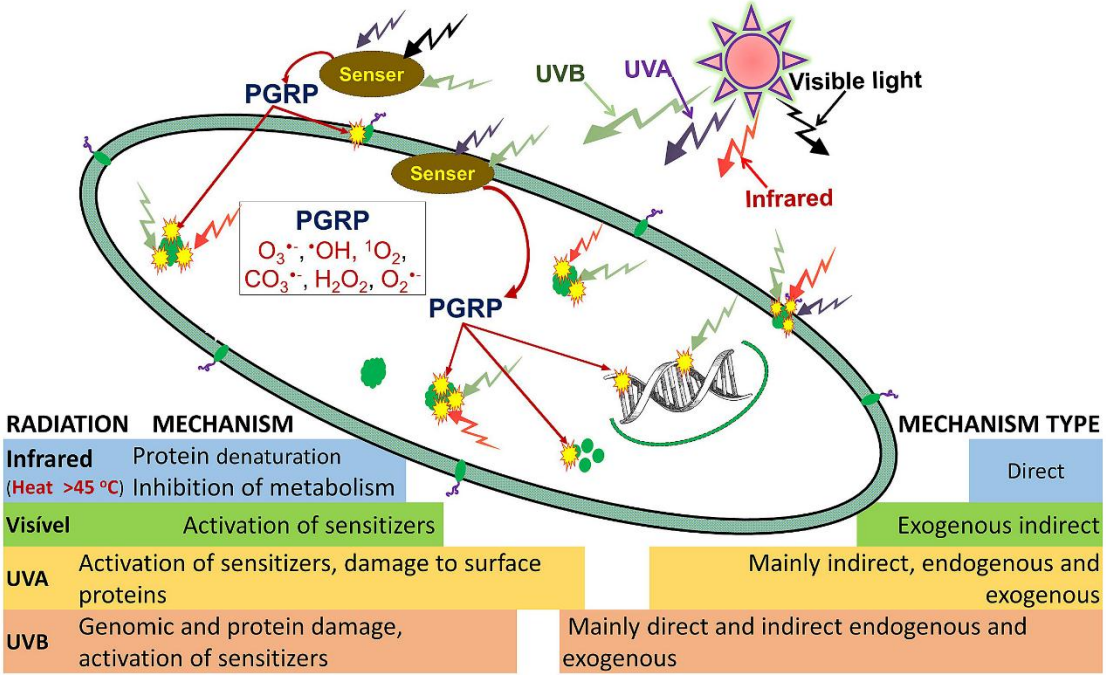

Fig. 7. Schematic representation of the pathways of synergistic demobilization of microbes by solar energy. In direct pathways, energy damages the biomolecule absorption site (yellow light). In indirect pathways, energy is absorbed by a sensitizer and induces the generation of photo-generated reactive products (PGRP) that damage the biomolecule site (yellow light) that has not absorbed energy. Proteins are represented in green [43].

To examine the capacity of high-pH conditioning as a disinfectant-free mohair for monitoring *L. pneumophila* and different pathogens, Pinel et al. [99] worked on a pilot-scale cooling tower with demineralized water was used. They realized one control test under standard full-scale operation implying sodium hypochlorite injection and tried 3 alkaline pHs of the cooling water: 9.0, 9.4 and 9.6. The experiments continued between 25 and 35 days. The *L.*

pneumophila analyses depicted substantial development at pH 9.0 and pH 9.4 yet was kept below detection limit (< 100 CFU/L) at pH 9.6 without disinfection. Most importantly, the findings correlated with the overall abundance of protozoa in the water samples but not directly with the relative abundance of specific reported protozoan hosts of *Legionella*. They concluded that high-pH conditioning ≥9.6 is seen as an efficient disinfectant-free cooling tower operation for monitoring pathogenicity, comprising *L. pneumophila*.

Delaedt et al. [100] studied electrochemical disinfection in order to kill *L. pneumophila* and *E. coli* in tap water. They spiked water with bacteria (10^4 CFU *E. coli* or *L. pneumophila*/mL) and passed it across an electrolysis cell (direct impact) or introduced bacteria into tap water after passage across such disinfection unit (remaining impact). The spiked tap water was totally disinfected, through passage across the electrolysis cell, surprisingly when only a residual free oxidant level of 0.07 mg/L is left (*L. pneumophila*). The remaining impact conducts to a full suppression of cultivable *E. coli*, if after reaction time at least a free oxidant concentration of 0.08 mg/L is still present. Identical circumstances diminish considerably *L. pneumophila*; however, a full eradication is not reached [100]. Furuta et al. [101] examined the DiaCell® towards *Legionella* contagion in different water types and below numerous running circumstances. Following the water matrix, *Legionella* could be totally neutralized with current densities as small as 50 mA/cm^2 with low short periods (<5 min). The higher the oxidant level in the electrolyzed water, the fastest is the *Legionella* demobilization following introduction. Bicarbonates in polluted water were recognized as outstanding supports for electrochemical disinfectants formation for killing *Legionella* without high chlorine level [101,102]. Feng et al. [103] tested the electrochemical disinfection of germinated brown rice (GBR) circulating water and cooling tower water carrying *Legionella*. The total aerobic plate counts in the treated GBR circulating water diminished considerably and *Legionella* bacteria were as well neutralized at a pulse voltage of 1.0 kV. The killing phenomenon was related to the synergistic impacts of the oxide anode, the electric field, and the radicals generated throughout the electrochemical application [103].

Using neutral electrolyzed oxidizing water (NEOW) is an encouraging technique for dealing with *L. pneumophila* in hot water systems and possesses the benefit of a low annual cost of production (0.02 V for 1 L of NEOW with an active chlorine concentration of 500 mg/L) and the maintenance of a device (~ 2000 V), which can be remotely controlled for pH and residual chlorine [104].

A mobile photoelectric point-of-use apparatus was suggested and its efficiency on pathogen demobilization established [105]. The apparatus uses a commercial teacup from which TiO_2 nanotube photoanodes were fabricated *in situ* and, with a small rechargeable battery powered 365 nm light emitting diode, was capable to reach 5-log demobilization of *E. coli* in 10 s and 2.6-log of *Legionella* in 60 s of treatment in model water samples. Treating natural water attained a 1-log bacteria demobilization following 30 s due to matrix effects. Such findings establish the capacity for illumination to ameliorate the performance of electrocatalytic surfaces [105]. Jeong et al. [106] confirmed the efficacious application of immobilized Ni/TiO_2 mesh in water disinfection applications, especially against *L. thermalis*. De Battisti et al. [107] proved that even *P. aeruginosa* and *Legionella* can be totally eliminated in the treated stream, thanks to the unique potential of the usage of the CabECO® reactor system to form biocidal agents such as ozone, $HOCl/ClO^-$, and chloramines.

Nakamura et al. [46] followed constant *Legionella* pollution of water faucets in a tertiary hospital in Japan. They concluded that controlling pollution, hyper-chlorination, monitoring temperature, and flushing water taps remain performant as a *Legionella* purge procedure; further, purge procedures have to be tried for performance and practicability at each facility [46]. Costa et al. [6] compared the performance of hyper-chlorination and the integration of hyper-chlorination and UV irradiation disinfection towards legionellae that are found in the water utilized to supply the therapeutic spa. Muzzi et al. [108] juxtaposed three various decontamination techniques by monitoring colony-forming unit count and number of hospital-acquired legionellosis cases and estimated the long-term impacts of the preventive procedures on the water pipes. They found that using shock disinfection and hyper-chlorination conducted to a reduction in contagion degree instantaneously following the measure; however, then it augmented again to the initial degree during sixty days. Both copper-silver ionization and ClO_2 disinfection depicted a stable and durable reduction in pollution degree. Concerning the deterioration of water pipes, efficient copper-silver levels provoked corrosion and calcification in water pipes [108]. Baron et al. [66] established the potential usefulness of high-throughput DNA sequencing to observe microbial ecology in water systems.

As an encouraging technique for large-scale disinfection without adding novel chemicals, hydrodynamic cavitation (HC) has appeared [109]. HC could efficiently generate sonochemistry by mechanical means. It forms extraordinary circumstances of pressures of ~1000 bar,

local hotspots with ~5000 K, and high oxidation (hydroxyl radicals) at ambient temperature. Such circumstances could form highly destructive impacts on pathogens in water. Further, ameliorating chemical reactions and mass transfers via HC form the synergism between HC and disinfectants or different physical treatment techniques. Sun et al. [109] discussed the basic concepts of HC and fresh expansion in HC disinfection. Employing inactivating *L. pneumophila* as an illustration, Šarc et al. [110] estimated the killing performance of a Venturi apparatus with 2 L of water at developed and super-HC circumstances. The suggested HC circumstance (500 kPa pump pressure) conducted to a modest reduction (23%) following 48 min of treatment, at the same time a diminution of ~99% was reached following 60 min under super-HC circumstances with a pump pressure of only 20 kPa.

Concerning biological treatment systems, the utilized techniques depict numerous restrictions that have to be more examined [111]. As an illustration, UV disinfection was found to be a performant technique to kill *Legionella* in treated wastewater. However, UV light leaves no remaining killing agent and *Legionella* are apt to recolonize reclaimed water systems. In addition, *Legionella* are apt to recondition the DNA deteriorated provoked by the UV irradiation, which implies that *Legionella* are not detectable instantly following the UV application, yet it manifests following a period of time or some kilometers downstream from the discharge point [111]. Ozonation has as well been observed as a performant disinfection process for *L. pneumophila* in urban wastewater [112]; still, ozone does not constitute a remaining level in water, consequently it cannot ban *Legionella*'s regrowth in reclaimed water systems. More interest should be addressed to the estimation of the most efficient killing agent in reducing *Legionella*'s regrowth in reclaimed water systems [16,33,113]. Suggesting novel and innovative wastewater treatment techniques would constitute practical solutions to avert the development of *Legionella* in wastewater treatment plants (WWTPs) [111,114]. To reduce the emission of polluted aerosols from industrial and urban WWTPs, particularly the ones with higher danger of *Legionella* colonization, numerous alternatives (e.g., roofing or air filtration) remain to be estimated to avoid future LD manifestations inside the WWTP or in the proximity regions [111].

6. Conclusion

This work defined microbiologically *Legionella* bacteria and presented a short history relating to their first discovery and following contagions, a brief description relating to their metabolism and physiology, a discussion of their clinical characteristics and their subsistence in the nature and growth in a biofilm, and a general examination of numerous technologies employed for their removal. The main points drawn from this work may be listed as below:

1. In terms of health impacts (manifestation of disease, and possibility of disease based on infection): (1) *Legionella* provokes legionellosis that possesses two forms: PF and LD. (2) PF is a comparatively mild, self-limiting, influenza-like disease. PF appears in otherwise healthy persons as pleuritic pain in the absence of pneumonic or multisystem manifestations. (3) PF possesses a brief incubation time, an elevated attack rate; however, a so small mortality ratio. (4) LD is a severe respiratory disease, and its contagion may persist for weeks to months. The symptoms of contamination comprise pneumonia with anorexia, malaise, myalgia, headache, rapid fever and chills, a cough, chest pain, abdominal pain and diarrhoea. Acute renal failure, disseminated intravascular coagulation, shock, respiratory insufficiency, coma and circulatory collapse are the major factors precipitating death. (5) LD possesses a small attack rate (1-6%), even if it has a mortality rate of around 10%. (6) LD remains more frequent in those with underlying diseases, smokers, the elderly, or the immunocompromised (for whom the prognosis is poor). (7) LD accounts for 1-4% of all cases of pneumonia, for all that rates as elevated as 30% have been noticed. The incidence of PF stays obscure [2].

2. Relating to exposure evaluation (channels of exposure and diffusion, existence in source water, ecological fate): (1) *Legionella* are omnipresent in the nature and have been isolated from different freshwater habitats comprising ground water, rivers, lakes and natural thermal pools. More important counts of *Legionella* have been reported in man-made water supplies (e.g., air-conditioning condensers, cooling tower effluent, humidifiers, nebulizers, drinking and hot water supplies, domestic and hospital showerheads, whirlpool spas, decorative fountains and vegetable misting machines). (2) The attachment of *Legionella* with freshwater protozoa in the aquatic environment is well-known. The organism multiplies intracellularly in protozoa and ciliates, particularly when water temperatures are high. Such association shields the bacteria versus dry conditions, extremes of temperature, and treatment with biocides. (3) Delivery of the agent to the respiratory tract in the form of water droplets (5-15 μm in diameter) works as the main channel for disease spread; aerosols carrying the organism create a main hazard element for nosocomial contagions and for those who are immunocompromised. (4) Individual-to-individual contagion of *Legionella* is not

considered to take place. (5) The contagious dose is undisclosed [2].

3. Concerning hazard reduction (potable water treatment and medical treatment): (1) *Legionella* is vulnerable to both heat and chlorine. Irregular temperature augmentations in the water supply of up to 60°C, also the application of chlorination techniques to furnish a constant 1-2 mg/L residual remain performant. Besides disinfection, remedial cleaning and flushing of water systems as well as the elimination of sediment from hot water tanks, combined with water treatment and constant water monitoring are requested. (2) It is crucial that when designing the plumbing systems of novel buildings and those undertaking amendment, dead space volumes are diminished, and sediment build-up and stagnation prevented because such conditions support development of legionellae, especially in biofilms. (3) The antibiotic most frequently suggested for legionellosis remains erythromycin; nonetheless, ciprofloxacin or rifampicin can be given. With PF most patients recuperate without specific therapy. (4) In immunocompromised persons the mortality rate for LD stays comparatively elevated, regardless of proper therapy [2].

4. In terms of the rapport between *Legionella*, the chemical parameters and the resident microbiota in cooling towers: (1) the origin of the water remains the prime element influencing the bacterial community of cooling towers. More investigations stay needed to explore if and how the water origin augments the probability of *Legionella* and *L. pneumophila* occurrence in cooling towers and how it could be dominated to decrease the related danger of LD. (2) The *Legionella* population itself is mostly impacted by alpha diversity, the concentration of *Pseudomonas*, dosages of chlorine, and the recurrence of chlorine injection. Persistent chlorination appears to aid microbial conditions that save the cooling towers versus *Legionella* colonization. (3) The occurrence of *Legionella* and *L. pneumophila* is linked with numerous taxa. Consequently, dominating the composition of the resident microbiota can be a different approach to aid diminish the occurrence of *Legionella* and *L. pneumophila* in cooling tower and lessen the danger of LD eruptions. More importantly, numerous taxa are uncultured or unclassified proposing that colonization of towers and probability of eruptions can be potentiated by as of yet uncharacterized interactions between *L. pneumophila* and several bacterial species. This justifies more study of the microbial relationships in water systems [4].

5. The spread of opportunistic pathogens (OPs) remains the most significant feature of microbial potable water quality. The levels and generation of disinfection by-products (DBPs) remain the most crucial feature of physicochemical potable water quality. Both OPs and DBPs in potable water considerably menace public health. The (re)growth of OPs and the production of DBPs in urban engineered water systems both closely correlate with the injections or concentrations of disinfectant residuals. Nonetheless, OPs and DBPs respond to disinfectant residuals frequently oppositely. An elevated residual concentration efficiently suppresses the (re)growth of OPs while intensifies the production of DBPs. Oppositely, a low or "detectable" disinfectant residual level decreases the generation of DBPs but could not stop OPs from thriving. We may need to comprehensively consider OP (re)growth and DBP generation while selecting a practical disinfectant residual dosage or level to guarantee that the overall or combined health risks of OPs and DBPs are minimum [35].

Abbreviation

CDM	Culture-dependent method
CFU	Colony-forming unit
COD	Chemical oxygen demand
DBPs	Disinfection by-products
DNA	Deoxyribonucleic acid
EMA	Ethidium monoazide
FCM	Flow cytometry
FM	Fluorescence microscopy
GBR	Germinated brown rice
HC	Hydrodynamic cavitation
LD	Legionnaires' disease
NEOW	Neutral electrolyzed oxidizing water
OPs	Opportunistic pathogens
PCO	Photocatalytic oxidation
PCR	Polymerase chain reaction
PF	Pontiac fever
PGRP	Photo-generated reactive products
PMA	Propidium monoazide
qPCR	Quantitative PCR
RT-qPCR	Reverse transcription qPCR
UV	Ultraviolet
VBNC	Viable but non-culturable
WWTP	Wastewater treatment plant

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Conflict of Interest

The authors declare that they have no conflict of interest

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