Hand in Hand approach by culture and PCR – a plausible solution for effective management of risks associated with Legionella in water samples

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Legionella in water distribution systems appear to be an unescapable threat to public health. The intensity of Legionellosis may vary from mild respiratory illness to potentially deadly pneumonia. Global incidences of Legionnaires disease outbreak has been raising over the years. Thus reliable analytical procedures play a crucial role in the evaluation of risk assessment and control of Legionella. Analytical methods involving traditional culture method and PCR based rapid methods are currently being used in water quality monitoring. While each method has its own advantages and limitations of underestimating and overestimating the occurrence of Legionella, we propose simultaneous application of culture and real time PCR for effective monitoring of Legionella in water distribution systems.

Background

Legionella in water distribution systems appear to be an unescapable threat to public health. Global incidences of Legionnaires disease outbreak has been rising (Whiley *et al.*, 2014) ranging from mild respiratory illness to potentially deadly pneumonia. A total of 64 *Legionella* species have been characterized to date of which *L. pneumophila serogroup* being the most commonly reported pathogen in more than 90% cases (Ditomaso *et al.*, 2014). The ubiquitous presence of the bacterium pave the way for an easy exposure to human to be an asymptomatic carrier or susceptible to mild / severe respiratory illness depending on the vulnerability of the individual. According to the available data from Centre for Disease Control (CDC, USA), about 9% of the Legionnaires’ disease cases are fatal (Dooling *et al.*, 2015). Water distribution systems are thought to serve as the primary source for *Legionella* occurrence. Factors like water temperature (20–45°C), presence of organic and inorganic substances also promote *Legionella* growth and/or infectious aerosol. Based on the available scientific publications, abatement of Legionella in water distribution system is best achieved by point-of-use methods, gatekeeper systems and by treating the entire water systems such as complex water distribution systems. Of the treatment methods available, Copper-silver ionization (Cloutman-Green *et al.*, 2019) and heat and flush are the methods that can treat the entire water system without forming toxic by-products.

Risk Assessment

Given the magnitude of the problem, quantitative microbial risk assessment (QMRA), a model originally developed to understand the consequences of microbial exposures in food was applied in the water distribution systems. The model relies on both laboratory and field data to identify an infection threshold for effective risk assessment and management. Application of QMRA in the case of *Legionella* is convoluted due to the fact that most of the investigations are linked to outbreaks. In addition, discrepancies in culture and qPCR analytical methods with varying risk scenario make QMRA less effective. Further research on better integrative understanding of mathematical frameworks for *Legionella* modeling could produce more rigorous risk prevention strategies (Hamilton and Haas, 2016).

Nevertheless, risk assessment implementation is part of the safe drinking water Guidelines proposed by World Health Organization (WHO) and a practical assessment is crucial for effective water quality management (Peter *et al.*, 2017). Many national governments have implemented evaluation programs in order to minimize risks associated with Legionella in water distribution systems.

For a reliable risk assessment, quantification of Legionella from the source is a prerequisite and hence analytical testing play a pivotal role in determining the risks. The technical Guidelines of European Working Group for Legionella Infections (EWGLI) stipulates a weekly monitoring on uncontrolled sites to monthly and quarterly sampling on systems where control levels of treatments are achieved (Whiley, 2017). In the United Arab Emirates (UAE), the code of practice for the inspection and cleaning of customer water storage tanks stipulates periodic cleaning and disinfecting and mandates quarterly lab testing to ensure the efficacy of water treatment systems in addition to other microbiological parameters for general cleanliness (RSB, 2015). Guidelines for the Control of Legionella in manufactured water systems in South Australia specify microbiological testing at least once in every 12 months by an accredited laboratory (Bedard *et al.*, 2016).

Detection and quantification of Legionella

The degree of risk is best evaluated when analytical methods yield accurate information on the real status of the organism of interest. In the case of *Legionella*, culture based isolation and enumeration remains the “gold standard” while methods like quantitative PCR and immunemagnetic separation (IMS) are also being used (Toplitsch *et al.*, 2018; Diaz-Flores *et al.*, 2015). Each method has its own advantages and limitations. For instance, traditional culture method offers the advantage of detecting viable cells but with longer incubation period while real time PCR and IMS techniques offer rapid detection of legionella with no reliable discrimination of live and dead cells. Nevertheless the real time PCR detects viable but non-culturable (VBNC) cells where the organism is metabolically active but cannot grow on culture media. Assessment of VBNC is very crucial as the organism regains virulence after resuscitation into culturable cells under suitable conditions (Li *et al.*, 2014).

Lab Testing benefits risk assessment or a burden?

The controversies surrounding the culture and quantitative PCR results interpretation, the complexities associated with sampling and infective dose of *Legionella* challenge the role of lab testing. For instance, culture method expresses the result as colony forming units (CFU) while it is Genomic units (GU) by PCR with no reliable correlation between these methods. Hence an International trial to quantify Legionella in artificial water systems by the culture and qPCR methods was initiated to develop an algorithm for the interpretation of qPCR results in routine monitoring or outbreak investigations with some threshold values for risk assessment action (Lee *et al.*, 2011). Recently, Lizana and coworkers (2018) developed and validated viability qPCR (v-qPCR), a method to detect only viable cells of Legionella in water samples and applied the conversion factor between v-qPCR and culture by dividing the value expressed in GU 28 times to obtain cfu estimation (Ditomaso *et al.*, 2015). The study established a reasonable estimation coherent with their theoretical cutting point of 1000 GU/L. But still, qPCR is not considered for the quantification of Legionella in standards, guidelines, and regulations (Caicedo *et al.*, 2019). Further insights are required to establish global consensus on its applicability in standard procedures.

Recommendations from Centre for Disease Control (CDC), do not include routine testing as a monitoring tool as the organism is thought to be present almost everywhere and potable water is considered as constant source of *Legionella*. Thus it was proposed to remove testing regime from all guidelines (Whiley, 2017).

Moreover, testing regime has not eliminated out breaks as water systems have complex engineering designs and multifaceted equipment to recirculate water, existence of Legionella to thrive will continue (Bassett and Balter, 2017). It is evident that we still do not have a clear picture on the role and efficiency of analytical testing on Legionella risk management. Nevertheless, it does not necessarily mean that analytical testing do not contribute to Legionella risk assessment and management. In order to evaluate the intensity and impact of risks, knowledge on the occurrence of legionella and its distribution is very crucial which can only be obtained through testing. In addition, lab testing provides an indication on the treatment regime so that unnecessary disinfection on Legionella free areas can be avoided. Furthermore, isolation of Legionella from the distribution system and their genomic characterization provide evolutionary dynamics over a period of time in different geographical locations. Such information offers meaningful insights on outbreak investigations and epidemiological studies.

Analytical grey areas can be minimized by simultaneous application of culture and real time PCR as it fulfills both regulatory requirements and rapid screening on Legionella free environments. Although VBNC cells do not pose greater risk since these cells cause infections only when they transit back into the cultivable state (Casini *et al.*, 2018), higher concentration are considered to be a risk for human health (Marinelli *et al.*, 2017).

One of the concerns in the application of both culture and real time PCR would be the cost. However, the cost of real time PCR has been reduced drastically in the recent times and it has become a routine screening analysis in many laboratories. Moreover, real time PCR analysis reduces the turnaround time on negative samples. Since the benefits of simultaneous application of culture and PCR outweigh the marginal cost incurred on evaluating the chances of *Legionella* occurrence, we propose to apply hand in hand approach by both culture and real time PCR for *Legionella* risk evaluation until other techniques demonstrate otherwise.

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