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Abstract

Legionella pneumophila was frst introduced to the world after an outbreak in 1976 when the attendees of the Philadelphia convention of the American Legion were afected by a kind of pneumonia named Legionnaire's Disease (LD). To date, the problem has not been resolved, and it has been reported many outbreaks over the years. The topic of Legionella outbreaks is one of the most active areas, thus encouraging researchers to focus on how they could efectively monitor the water sources to prevent the risk of an outbreak and high mortality. Research into solving this problem and establishing a state of the art method is already in progress. This paper is an overview of *legionella* detection over the years. What happened to *Legionella* through the past challenging decades. Is there any certifed reference method with a functionalized penetration into society for this controversial microorganism or a cutting-edge scenario ofering a constructive solution to eliminate the potential risk of the outbreaks, also early identifcation and source tracking?

Keywords: Waterborne diseases; Microbiology

Introduction

Legionella pneumophila was rst introduced to the world a er an outbreak in 1976 when the attendees of the Philadelphia convention of the American Legion were a ected by a kind of pneumonia named Legionnaire's Disease (LD). To date, the problem has not been resolved, and it has been reported many outbreaks over the years. e topic of *Legionella* outbreaks is one of the most active areas, thus encouraging researchers to focus on how they could e ectively monitor the water sources to prevent the risk of an outbreak and high mortality. LD outbreaks are associated with the increased risk of contamination of the cooling towers, drinking water supply systems, spa pools, and decorative fountains [1]. In general, therefore, it becomes a growing public health concern worldwide. *Legionella*, however, is an obligate, intracellular,

Legionella through the past challenging decades. Is there any certi ed reference method with a functionalized penetration into society for this controversial microorganism or a cutting-edge scenario o ering a constructive solution to eliminate the potential risk of the outbreaks, also early identi cation and source tracking? [2]

Conventional Routine Techniques

Microbiological Investigations

Commonly referred to as the gold standard; Culture is still a traditional common method performing by many laboratories. Bu ered Charcoal Yeast Extract (BCYE) agar supplemented with an absolute requirement, L-cysteine, and α-ketoglutarate, with or without antibiotics, has been known as a standard medium for the isolation and estimation of *Legionella*. Since L-cysteine is a signi cant growth enhancer, an organism that grows on BCYE supplemented with L-cysteine is most likely classi ed as *Legionella spp*. However, culture su ers from a lack of ability to detect Viable But Non Culturable (VBNC) and overgrowth of microbial ora, which are the main obstacles performing culture as a double-edged sword. Likewise, culture is time-consuming, requiring prolonged incubation time about 14 days at 37Co with 5% CO2. Bacterial colonies appear to be gray, glistening with a 3-4 mm diameter. e Culture technique is not able to detect *Legionella* within amoeba [3]. Furthermore, it is not worthwhile for all *Legionella* species because they are not cultivable; it proved ine cient. Another drawback of this technique is that culture is not sensitive enough for the low counts of bacteria and indicates the lowest sensitivity than PCR and UAG test. e sensitivity of culture has been reported by approximately 10%-80%, depending on the samples' nature, technician skills, and antibiotic therapy. Besides, pre-treatment (heat and acid) for water samples before the culture is laborious and contributes to missing *Legionella's* even minimum level. e standard samples for culture are respiratory specimens, BAL and sputum, and water. Apart from the poor survival and yield of *Legionella* in respiratory secretions, most of the patients with Legionnaires' disease are not able to routinely produce a sputum sample. Despite all limitations, culture has been in accordance with the International Organization for Standardization (Water quality-Enumeration of *legionella*-ISO 11731:2017). To make a long story short, the generation of culture-independent techniques are considerably demanding; covering the shortages of classical means, also opening up a new eld of research. Due to the proper bioinformatics studies and primer/probe designs in nucleic acid-based methods, the speci city is around 100% and sensitivity is equal or more than culture [4].

PCR (Polymerase Chain Reaction)

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the inability to detect species/serogroups other than *L. pneumophila* serogroup 1. It should be noted that one of the disadvantages is that, it fails to identify other *Legionella* species while detection of serogroup 4 and 6 are vitally important equal to serogroup 1. UAT is commercially available in two Binax Enzyme Immuno Assay (EIA) and Immuno Chromatographic Test (ICT). Since the UAT is particularly useful and has a number of attractive features, further attempts should be undertaken to cover *legionella* species other than serogroup 1. us, care is required when interpreting results. Antibody-based approach is of another type of non-invasive method. One downside regarding this methodology is the cross-reactivity between species. Besides, it has a poor performance during the acute phase in serum sample and is rather disappointing as a result of emerging new molecular and standardized culture methods. Direct Fluorescent-Antibody Assay (DFA) is applied for qualitative identi cation and typing of *Legionella* [9].

Real-time PCR

In the midst of the evolution era in biotechnology, real-time PCR claimed to be a molecular gold standard and represented a leap forward in all elds of research and innovations. It provides a profoundly insight into the kinetics of reactions, also surpassing other PCR models. When compared to other experiments, Quantitative real-time