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Serotype to genotype: The changing landscape of listeriosis outbreak investigations

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ABSTRACT

The classical definition of a disease outbreak is the occurrence of cases of disease in excess of what would normally be expected in a community, geographical area or time period. The establishment of an outbreak then starts with the identification of an incidence of cases above the normally expected threshold during a given time period. Subsequently, the cases are examined using a variety of subtyping methods to identify potential linkages. As listeriosis disease has a long incubation period, relating a single source or multiple sources of contaminated food to clinical disease is challenging and time consuming. The vast majority of human listeriosis cases are caused by three serotypes, 1/2a, 1/2b, and 4b. Thus serotyping of isolates from suspected foods and clinical samples, although useful for eliminating some food sources, has a very limited discriminatory power. The advent of faster and more affordable sequencing technology, coupled with increased computational power, has permitted comparisons of whole *Listeria* genome sequences from isolates recovered from clinical, food, and environmental sources. These analyses made it possible to identify outbreaks and the source much more accurately and faster, thus leading to a reduction in number of illnesses as well as a reduction in economic losses. Initial DNA sequence information also facilitated the development of a simple molecular serotype protocol which allowed for the identification of major disease causing serotypes of *L. monocytogenes*, including a clade of 4b variant (4bV) strains of *L. monocytogenes* involved in at least 3 more recent listeriosis outbreaks in the US. Furthermore, data generated using whole genome sequence (WGS) analyses was successfully utilized to develop a pan-genomic DNA microarray as well as a single nucleotide polymorphism (SNP) based analysis. Herein, we present and compare, the two recently developed sub-typing technologies and discuss how these methods are not only important in outbreak investigations, but could also shed light on possible adaptations to different foods and environments.

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Contents

1. Introduction

Human listeriosis is a major foodborne disease affecting approximately 1600 people per year in the USA (Scallan et al.,

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2011). The invasive disease, caused by *Listeria monocytogenes*, is characterized by septicemia, meningitis, and abortion. Infections result in >95% hospitalization and 15–20% death rates. *L. monocytogenes* has also been involved in several febrile gastroenteritis outbreaks (Norton and Braden, 2007). The susceptible population for invasive listeriosis includes the very young, elderly, pregnant women, and immune-compromised individuals. However, occasional illnesses are found in apparently healthy individuals, as seen in the 2015 caramel apple outbreak, where cases of young individuals (5-15y), without any reported underlying illness noted (CDC, 2015). As the onset of invasive listeriosis could be a few days to several weeks, it can be very difficult to identify the source of infection in a timely fashion. The initial outbreak investigation depends on the identification and reporting of cases followed by epidemiological investigations to identify the possible source of the contamination. Thus, it is not surprising that many of the sporadic, and a few outbreak cases, remain unsolved in terms of locating the contaminated food. Although a strong epidemiological link is crucial to connect a particular food exposure with disease, the complete establishment of the food-disease link requires isolation of *L. monocytogenes* m from food, clinical, and environmental sources followed by the establishment of close genetic relatedness among these isolates through a variety of biochemical, serological, and genomic techniques (Datta et al., 2013). Thus, it is imperative that molecular techniques are sensitive, rapid, and reproducible, and that the data are easily interpretable with a high degree of discriminatory power and confidence.

L. monocytogenes (Lm) is a gram-positive, non-spore forming bacterium that is ubiquitously present in the environment and reported to have wide range of adaptability to overcome different stresses. One of the unique properties of Lm is the ability to grow at refrigeration temperatures, provided other conditions are appropriate, e.g. A_w, pH and the availability of nutrients (Lado and Yousef, 2007). The genus *Listeria* is comprised of about 17 species, some of which were recently reported (Orsi and Wiedmann, 2016). Of all these species, Lm is the only species that causes the vast majority of human listeriosis. *Listeria ivanovii,* which contains most of the known virulence genes, causes animal listeriosis, but only very rarely results in human infections (Guillet et al., 2010). As all Lm are considered pathogenic; it is important that strains are further characterized by a variety of subtyping tools so that the genetic footprint of outbreak strains can be identified and matched with isolates from foods and/or environment in order to establish links between disease and a particular food.

2. Serotyping

Serotype analysis, based on somatic and flagellar antigens, is the most basic typing method for Lm. The members of the major species of *Listeria*, including Lm, can be divided into 13 serotypes (Seeliger and Hohne, 1979). Out of these 13 serotypes, most of the human diseases are caused by only three serovars, 1/2a, 1/2b, and 4b; serovar 4b is most associated with outbreaks and responsible for about 50–60% of clinical cases (Liu, 2006, 2008; Cartwright et al., 2013). Similarly, the majority of strains from foods and the food processing environment also belong to serotypes 1/2a, 1/2b and 4b; although relative abundance of these three serotypes is different than that seen in clinical cases (Sauders and Wiedmann, 2007). Given such a narrow distribution of serotypes among clinical, food and food processing environments, serotype analysis is not often useful in the establishment of an epidemiological link to the contaminated food source. The standard serotyping approach also suffers from being laborious, time consuming, and subjective. An abbreviated version of serotyping, also known as a slide agglutination test, where isolates are classified as type 1, type 4, or non-typable, has been used for many years (Hitchins et al., 2016). In recent years, several PCR-based methods have been proposed to classify Lm into different serogroups (Borucki and Call, 2003; Doumith et al., 2004a,b). These methods utilize unique genomic regions of the different serotypes for the development of primers specific to each of the different serogroups. Using one such PCR-based method (Doumith et al., 2004a) in conjunction with a type 1 and type 4 specific slide agglutination assay, Burall et al. (2011) showed that the majority of the Lm isolates can be grouped as 1/2a, 1/2b, 2c, 3a, 3b and 4b (Table 1). The PCR-based serotype assay utilizes the presence or absence of specific amplicons generated by a standard PCR or by a real-time PCR method (personal communication). The PCR based method was instrumental in finding a small fraction of 4b strains that contained a 1/2a specific amplicon, lmo0737 (Table 1). This group of strains identified as serotype 4b using an Ag-Ab test, however, it contains a 6.3 kb DNA insertion that is specific to lineage II strains, e.g. serotypes 1/2a, 1/2c strains. These strains, termed 4bV or IVb-v1, have been reported from different parts of the world and have been found to be associated with foods and the environment, and have caused outbreaks as well as sporadic listeriosis (Huang et al., 2011; Leclercq et al., 2011; Lee et al., 2012; Burall et al., 2014, 2016; Camargo et al., 2016). Milillo et al. (2009) compared the genetic makeup of several 1/2a and 4b genomes and found that 1/2a genomes contain four DNA fragments, approximately 5.1 kb, 6.5 kb, 5.0 kb and 3.8 kb in size, that are not present in 4b strains. Out of these four fragments, the 4bV strains harbors only a 6.3 kb (part of the 6.5 kb fragment) region. Although the significance of this fragment, containing several putative sugar metabolism genes, in both the lineage II and lineage I backgrounds is not understood (Milillo et al., 2009), it is interesting to note that a clade of 4bV strains has been implicated in four recent listeriosis outbreaks in the USA (Burall et al., 2017). One of these outbreaks, associated with caramel apples, included individuals, ages 5–15 years, with no known underlying immunodeficiencies (CDC, 2015). Thus, it appears that, while for most of the listeriosis cases, serotype analysis may be of limited use, in some situations, e.g. outbreaks associated with unusual serotypes like 3a or 4bV strains (Table 2), it could provide an early lead during outbreak investigation including grouping of the isolates for further downstream analysis. It is, however, interesting to note that there has been a clear shift in the distribution of serotypes in listeriosis outbreaks. While 4b was linked to the major outbreak serotypes prior to 2000, serotypes 1/2a, 1/2b and more recently 4bV strains have been found to be associated with the listeriosis outbreaks in the USA (Table 2) (Cartwright et al., 2013).

3. Pulsed Field Gel Electrophoresis based subtyping

As serotyping has very limited use in listeriosis outbreak

Table 1
Combination of a slide agglutination assay and a PCR assay for Lm serotyping.

Slide Agglutination		PCR Amplicon				Strain Serotype
Type 1	Type 4	lmo1118	lmo0737	ORF2110	ORF2819	
+	−	−	+	−	−	1/2a
−	−	−	+	−	−	3a
+	−	−	−	−	+	1/2b
−	−	−	−	−	+	3b, 7
+		−	+	−	−	1/2c
−	−	−	+	−	−	3c
−	+	−	−	+	+	4b, 4d,4e, 4 ab
−	+	−	+	+	+	4bV
−	−	−	−	−	−	4a, 4c

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