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A simple approach to obtain comparable *Shigella sonnei* MLVA results across laboratories

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ABSTRACT

Multilocus variable-number tandem repeat analysis (MLVA) is a promising subtyping tool to complement pulsed-field gel electrophoresis for discriminating closely related strains of some monomorphic organisms, including *Shigella sonnei*, which is one of the major foodborne pathogens. However, MLVA results are usually difficult to compare directly between laboratories, impeding the application of MLVA as a subtyping tool for disease surveillance and investigation of common outbreaks across regions or countries. It has long been a big challenge in seeking an approach that can be implemented to obtain comparable MLVA results across laboratories. By implementing a panel of calibration strains in each participating laboratory for data normalization, the MLVA results of 20 test strains were comparable even though some analytical conditions were different among the laboratories. This approach is simple, protocol independent, and easy to implement in every laboratory, and a small calibration set is sufficient to generate mathematical equations for accurate copy number conversion.

Introduction

Multilocus variable-number tandem repeat analysis (MLVA) is a well-known promising tool for subtyping of a number of organisms. Although MLVA is very organism-specific, at the level of serovars or even clones within a species (Chiou, 2010; Filliol-Toutain et al., 2011; Wang et al., 2009), it is rapid, easy to manipulate, and high throughput and is more powerful than pulsed-field gel electrophoresis (PFGE) in discriminating closely-related strains of some bacterial organisms. PFGE is a discriminatory subtyping method and has been adopted as the standard subtyping tool for PulseNet International, a molecular subtyping network for surveillance of foodborne diseases at the global level (Swaminathan et al., 2006). In contrast to MLVA, PFGE is quite universal. It can be applied to almost all bacterial species and a PFGE protocol is applicable to all the species within the same genus. However, PFGE is sometimes insufficient in discriminating strains of monomorphic organisms or strains within an endemic clone (Chiou et al., 2010; Liang et al., 2007; Torpdahl et al., 2013). MLVA is therefore considered as an alternative subtyping tool to complement PFGE in discriminating closely related strains for investigation of disease outbreaks and operation of a disease surveillance network across regions or countries. To accomplish the purposes, MLVA results obtained in different laboratories have to be comparable.

The main procedures of MLVA include PCR amplification of DNA fragments of alleles, measurement of fragment sizes with appropriate size markers by electrophoresis, and conversion of the measured fragment sizes into copy numbers of VNTR repeats or lengths of VNTR area (repeat array). The sizes of fragments measured in different laboratories may be greatly discrepant as a consequence of the use of different primer sets, labeling dyes, size markers, and electrophoresis platforms. It has long been a big challenge in seeking an approach that can be implemented to convert measured fragment sizes of alleles into comparable copy

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numbers or repeat array lengths across laboratories. Hyytia-Trees et al. (2010) conducted a multilaboratory validation study of a standardized MLVA protocol for *Escherichia coli* O157 to test the reproducibility of the protocol and to construct a common fragment size range table for each of the capillary electrophoresis platforms. Only 2 out of the 10 participating laboratories were able to generate correct MLVA types for the 50 strains tested. In 2010, we conducted a multilaboratory comparison study of a *Shigella sonnei* MLVA protocol. The results generated in 3 participating laboratories were not fully comparable. On May 2011, an International MLVA Harmonization Meeting was held in Copenhagen, Denmark. At the meeting, a concept that recommended to implement a panel of calibration strains in MLVA analysis for copy number conversion was proposed to solve the problem. After the meeting, Larsson et al. (2013) conducted a multilaboratory evaluation of the *Salmonella enterica* serovar Typhimurium MLVA scheme and we performed a second-run multilaboratory comparison study on the *S. sonnei* MLVA scheme, with a panel of calibration strains in each study. Here, we report the results of two-runs of multilaboratory comparison studies on the *S. sonnei* MLVA scheme. Our study indicates that comparable MLVA results are able to obtain across laboratories through data normalization with the use of an appropriate panel of calibration strains in each individual laboratory.

Materials and methods

The first multilaboratory comparison study

Three national public health laboratories in three Asian countries, Lab 1–Lab 3, participated in the first-run multilaboratory comparison study that was conducted in the second half of year 2010. DNA of bacterial strain was prepared using a commercial kit (Genomic DNA Mini Kit, Geneaid, Taiwan). All the participating laboratories analyzed the same batch of DNA samples from 30 *S. sonnei* strains, which were taken as the calibration strains in the second-run multilaboratory comparison study. For each strain, 8 VNTRs, SS1, SS3, SS6, SS9, SS10, SS11, SS12, and SS13 (Liang et al., 2007), were analyzed. A common protocol was followed, including the use of the same dye-labeled primer set (Table 1), size markers (GeneScan™ 500 LIZ® Size Standard, Applied Biosystems, Inc.), and capillary electrophoresis instrument, 3130xl Genetic Analyzer (Applied Biosystems, Inc.). The raw data of fragment lengths measured in the participating laboratories were collected for analysis.

The second multilaboratory comparison study

Four laboratories, Lab 1, Lab 2, Lab 4, and Lab 5, participated in the second-run comparison study that was conducted in the second half of 2012. The two new participants included one university laboratory and one national reference laboratory in an Asian country and a European country. All the participants analyzed the same batch of DNA samples from 30 calibration strains and 20 test strains with known copy numbers of the alleles in 8 VNTRs. Table 2 listed the alleles present in the 30 calibration strains and the 20 test strains. No protocol was suggested but all the laboratories used the same model of electrophoresis instrument, Applied Biosystems' 3130xl Genetic Analyzer. Three laboratories used the primer set listed in Table 1, while Lab 2 used its own in-house primer set. Lab 2 and Lab 5 used GeneScan™ 600 LIZ® size markers, while Lab 1 and Lab 4 used GeneScan™ 500 LIZ® size markers. Eight VNTRs, SS1, SS3, SS6, SS9, SS10, SS11, SS13, and SS23, were analyzed. On this study, SS12 was replaced by SS23 because some of the 30 strains contained two SS12 copies in genomes and the participants had difficulty in amplifying the locus. SS23 is more diverse than SS12 in two clonal groups of *S. sonnei* (Filliol-Toutain et al., 2011). The raw

Table 1
Primers and the information for calculation of copy number of repeats in 9 *Shigella sonnei* VNTRs.

VNTR	Repeat, bp	5′ flank, bp	5′ flank	Repeat sequence	3′ flank	3′ flank, bp	Number of repeats[b]	Dye-primers
SS1	7	27	TCGACGGAAA	ATCGCGCC	ATCATCACAG	98	(X-125)/7	VIC-TTGCCAGTACACCTCACTCG; GCGTCCGCGTTAATATCACT
SS3	7	88	AAAACTCGTC	CATTCAA	CAAGGAAAGA	26	(X-114)/7	6-FAM-CTGGGAGATGAACACGGAGGA; ATGCCAGCGACAAGTTTCTT
SS6	7	42	CTCGCGATCAT	AGAAAGC	GATGAACTTT	110	(X-152)/7	NED-GACTCGCTAAACGCTTGCTT; GGGAAATAGACGCGGACCTTT
SS9	6	83	CACCTTCGCGG	TGCCAGG	TTTAGCTTCC	78	(X-161)/6	VIC-CGCAATCAGCAAAACAAAGA; GCGATGCTGCGAAAAACTGAT
SS10	6	154	AAGCGTTACC	AGACGGA	AGATCTAAAT	27	(X-181)/6	6-FAM-ACGGTGGGCTTTCTCTACCT; GCGAGGGGGAGATCAGTATT
SS11	6	103	ACGCAGCCAG	AGTCAG	AGCAGGATAA	72	(X-175)/6	NED-CTGGTCCGGGACATTATCG; CTGTTTCAGCGGTCTCTTCC
SS12	9	81	TCGCACCCAG	CACCGGGTTA	AGCATAGGTA	89	(X-170)/9	NED.GCTGTAGGCACGGAAAAGAA; TGGATATTGTGCAGGGTTCA
SS13[a]	6	73	CGGCGACGGC	GCTGGT	ACAGAATATT	145	(X-218)/6	VIC-AGACGCTGGCTTATGACGAT; GCCACTGTCCTGAATGGTCT
SS23[a]	16	28	ACGGCAGTCG	GTTAACGCTTACCTCC	AATCTGCTGA	104	(X-132)/16	PET-CTGGCTTAATGGCTACATAC; CGCATGAGCGTGTTGTAATG

[a] The first repeat unit assigned for SS13 is GCTGGG instead of GCTGGT and SS23 is GTTAACGCTTACCTTT instead of GTTAACGCTTACCTCC.
[b] X designates the real size of the analyzed fragment.