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Sequencing of targeted Neisseria meningitidis genes directly from clinical specimens

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Background

Neisseria meningitidis (Nm) is a leading cause of bacterial meningitis and sepsis worldwide. Culture-dependent methods are commonly used for pathogen detection and characterization, but they can be time consuming and labor intensive. We previously developed an enrichment and sequence-based culture-independent method, named “selective whole genome amplification sequencing (SWGAseq)”, and have utilized it for obtaining Nm’s whole genome sequences directly from clinical specimens, such as cerebrospinal fluids (CSFs) that contain low bacterial load, thus eliminating the need for culture. In this present work, Amplicon sequencing (AmpSeq), a sequence-based culture-independent method with a different principal of detection has been developed to extract the genomic data from bacterial genomes in Nm-containing CSFs that failed to be sequenced using SWGAseq.

Aim/Methods

Here we have explored the potential use of AmpSeq to “rescue” and obtain the genomic data from these failed-to-be-sequenced bacterial genomes, thereby enhancing molecular meningococcal surveillance. AmpSeq utilizes the multiplex PCR-based library preparation technology that targets 13 genes in the Nm genomes essential for molecular typing [7 Multilocus Sequence Typing (MLST) house-keeping genes (abcZ, adk, aroE, fumC, gdh, pdhC and pgm), 3 fine typing genes (PorA, PorB and FetA), and 3 major serogroup B meningococcal (MenB) vaccine antigen genes (FHbp, NhbA, and NadA)].

Results

Studies using Nm isolates (n=9) demonstrated identification of 100% of the total of 13 meningococcal genes of interest. Among all tested CSFs (n=38), that failed sequencing using SWGAseq (n=25), we obtained

genomic data for 24% (6/25) of samples for MLST genes, 100% (25/25) for fine typing genes and 88% (22/25) of MenB vaccine antigens. The success rate for MLST genes, fine typing genes and MenB vaccine antigens was respectively, 85% (11/13), 100% (13/13) and 85% (11/13) among the samples that were amplified successfully by SWGA. The overall success rate for both SWGA success and failed samples was 45% (17/38) for MLST genes, 100% (38/38) for fine typing genes and 87% (33/38) for MenB vaccine antigens.

Conclusions

Thus, AmpSeq can serve as an adjunct method for obtaining important genomic information from Nm genomes when other sequencing-based approaches fail to provide the desired outcome.