Metagenomic Characterisation of Vaginal Flora and the Relationship of Bacteriophage, *Bifidobacterium* and *Oenococcus* with Bacterial Vaginosis in HIV Infected Women

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ABSTRACT

Bacterial vaginosis (BV) is a highly prevalent condition and the most common cause of abnormal vaginal discharge. Despite the high prevalence of BV and associations with other infections such as HIV-1 and obstetric and gynecological morbidity associated with it, the etiology of BV is still not clearly understood. It has been hypothesized that some unknown influences cause a significant decline in hydrogen peroxide producing lactobacilli allowing the overgrowth of anaerobic bacteria. The organisms implicated with BV have mainly been isolated by culture methods. The present study was done in two phases where the first was a pilot study done in California with the aim of determining the novel and predominant culturable and unculturable microorganisms associated with BV. To achieve this, micro-array and shot-gun sequencing techniques were used. Simpler techniques were also developed in phase one part of the study. The second phase was done in Kenya with the aim to determine the prevalence of BV among women infected with HIV and determine the association of the condition with bacteriophages and the specific microorganisms identified in phase one study. This was achieved using multiplex and simplex PCR with organism's specific primers. The pilot study in California identified two main organisms that had a relationship with BV presence or absence. *Oenococcus oeni* were present only in BV negative samples while *Bifidobacterium* species were significantly associated with BV positive samples ($p=0.0472$, Fisher's exact test). Bacteriophage type A2 was detected in 2 of 14 (14%) BV negative samples. Unculturable bacteria were over 90% of the total bacteria identified by sequencing. In Kenya, BV prevalence showed a declining trend while CD4 cells count increased with visit count. *Oenococcus oeni* was not detected in any sample. Bifidobacteria were present in 39/250 (16%) of BV positive and in 19/250 (8%) BV negative samples.

Though higher in BV positive the difference was not statistically significant ($p>0.05$; Chi-square). Bacteriophages Bradley types A1, A2, B1 and B2 were detected in BV positive and negative samples. Bacteriophage Bradley type B2 was detected at significantly higher rates in BV positive samples than in BV negative samples ($p<0.05$; Fisher's exact test). The use of different methods; shot-gun sequencing versus specific PCR may not have contributed to the differences of *Oenococcus* detection since, both methods are known to have high sensitivities with detection limits of $1\ \mathrm{pg}\ \mu\mathrm{l}^{-1}$ chromosomal DNA having been reported. Identification of *Oenococcus* and unculturable bacteria confirms that shot gun sequencing is an appropriate technique for identification of novel and unculturable organisms associated with BV. In addition, detection of BV associated organisms by PCR provides an effective screening method for BV. *Bifidobacterium* species are variably associated with presence or absence of BV and phylogenetic analysis shows some are close to BV related organisms while some are not. This indicates the likelihood that there exists variant species of Bifidobacteria. This study therefore concludes that novel and unculturable bacteria are the largest population of microorganisms in the vagina of women with and without BV.