B175 The Characterization and Persistence of Vaginal Bacteria Under Fingernails

An Truong, BS, 2957 Bowery Lane, San Jose, CA 95135; George Sensabaugh, DCrim, University of CA, Berkeley, School of Public Health, 50 University Hall, MC 7360, Berkeley, CA 94720; and Cassandra Calloway, PhD, Children's Hospital Oakland Research Institute, 5700 Martin Luther King Junior Way, Oakland, CA 94609*

The goal of this presentation is to inform attendees regarding the usefulness of bacterial flora in determining the biological source for vaginal contact in sexual assault investigations.

This presentation will impact the forensic science community by demonstrating that bacterial flora could be characterized according to their genera and evaluated to determine if there was vaginal contact in sexual assault investigations, which could provide more weight to sexual assault biological evidence.

For sexual assault cases involving digital penetration, probative DNA evidence from the victim's vaginal fluid could be under the suspect's fingernails. This type of DNA evidence can establish a direct link from suspect to the victim; however, the suspect could argue that the accumulation of the victim's DNA under the suspect's fingernails was due to casual or daily contact with the victim. A solution to this problem is to identify the body fluid as vaginal fluid using the presence of *Lactobacillus* bacteria species that are found natively in the vagina. Previous research has shown that it is possible to identify vaginal fluid using the 16-S ribosomal RNA (rRNA) gene of *L. crispatus, L. iners, L. gasseri,* and *L. jensenii.* Also, 16-S rRNA can be used to identify bacteria that are associated with a specific part of the human microbiome, such as the human skin. Resident flora that naturally occur on hands include *Staphylococcus*, *Proteus*, *Klebsiella*, and *Acinetobacter*. Further research has shown Next Generation Sequencing (NGS) technology can aid in 16-S rRNA sequencing and classifying bacteria associated with the vagina or skin. The goals of this project are: (1) to characterize the normal bacterial flora found underneath fingernails, in the vagina, and underneath fingernails following digital penetration; and, (2) to study the persistence of vaginal bacteria underneath fingernails following digital penetration.

In this study, 80 samples were collected from four couples (AAAB, BABB, CACB, and DADB) at designated time points (baseline, 0hr, 6hr, 12hr, 18hr, and 24hr) from underneath fingernails after digital penetration. The DNA was extracted, then the 16-S rRNA gene was targeted and amplified. The samples were then analyzed by NGS. A statistically significant difference in *Lactobacillus* frequencies when comparing source (experimental vs. control) was observed using a paired *t*-test (p=<0.0001). Higher frequencies of *Lactobacillus* were observed in experimental samples ranging from 0%-99.6% with a mean of 63.8%. In two couples, AAAB and CACB, the *Lactobacillus* populations were on average 6-11 times higher in experimental samples than control samples. The results were replicated in couple CACB and the experimental samples from that couple were predominately *Lactobacillus* even up to 24 hours ranging from 63.2%-99.6%. Also, a statistically significant difference in *Staphylococcus* frequencies when comparing source (experimental vs. control) was observed using a paired *t*-test (p=0.0046). Higher frequencies of *Staphylococcus* were observed in control samples ranging from 0%-97% with a mean of 41.9%. In two other couples, conversions from a predominately *Lactobacillus* population to predominately *Staphylococcus* population were observed after 6-12 hours.

Based on the results of this small study, it is possible to detect *Lactobacillus* at high frequencies after 24 hours; therefore, *Lactobacillus* could be used a biomarker to determine vaginal contact since a *Lactobacillus* population of a minimum frequency of 27% can suggest vaginal contact; however, a failure to detect *Lactobacillus* does not indicate the absence of prior vaginal contact.

Vaginal Bacteria, Sexual Assault Investigation, Next Generation Sequencing

*Presenting Author