RESISTANCE, CLONALITY AND CLINICAL ASPECTS ASSOCIATED WITH STAPHYLOCOCCUS AUREUS SAMPLES ISOLATED FROM COLONISATION SITES OF PAEDIATRIC AND ADOLESCENT PATIENTS INFECTED BY HUMAN IMMUNODEFICIENCY VIRUS

Introduction
HIV-infected patients are in a high-risk group to develop *Staphyloccocus aureus* infections. From 6% to 20% of these individuals have presented colonisation by methicillin resistant isolates (methicillin resistant *S. aureus* "MRSA"). In addition, *S. aureus* isolates may carry genes encoding the Panton Valentine leukocidin (PVL), responsible for lysing leukocytes. This study aimed to detect and characterise *S. aureus* isolates from nares, oropharynx and saliva from paediatric and adolescent patients infected with HIV enrolled in a public paediatric outpatient clinic, between 2014 and 2015.

Methods
The *S. aureus* identification was conducted after cultivation of specimens on mannitol salt agar, using conventional tests. The determination of antimicrobial susceptibility was performed by disk diffusion test, while the minimum inhibitory concentration (MIC) by the E-test was evaluated for mupirocin. PCR was used to detect the PVL genes and to determine the SCCmec types, while the PFGE technique was used for analysis of clonality.

Results
Among 100 patients included in the study, 68 (68%) presented *S. aureus* colonisation, and 15 (22%) of them were colonised by MRSA isolates. Colonisation by MRSA isolates was detected in the nares (17.6%), saliva (10.2%) and oropharynx (8.8%) of the patients. Among the 107 *S. aureus* isolates, the highest percentage of resistance was 26.1% for erythromycin, followed by cefoxitin (23.4%), gentamicin (4.7%) and teicoplanin (3.7%). All MRSA isolates carried the SCCmec IV and the PVL genes were found in 26 isolates of *S. aureus*. PCR was performed by disk diffusion test, while the minimum inhibitory concentration (MIC) by the E-test was evaluated for mupirocin. PCR was used to detect the PVL genes and to determine the SCCmec types, while the PFGE technique was used for analysis of clonality.

Conclusion
The study confirms the high frequency of colonisation by *S. aureus* in paediatric and adolescent patients with HIV. Besides the high rate of colonisation in nares over than a third of the patients presented colonisation in the oropharynx and/or saliva, important aspects to be considered in the control and prevention of infections caused by *S. aureus* isolates in HIV-positive individuals.

MICRONA EXPRESSION INDUCED BY VAGINAL MICROBIOTA CONTROLS CELL PROLIFERATION

Introduction
Bacterial Vaginosis (BV) is a condition of the human vagina characterised in part by a paucity of *Lactobacillus* spp. and the presence of a wide array of strict and facultative anaerobes such as *Gardnerella vaginalis* and *Atopobium vaginae*. BV is associated with the acquisition of sexually transmitted infections such as HIV and *Chlamydia trachomatis.* Host microRNAs (miRNAs) are an uncharacterized factor that may control host cellular responses to *Lactobacillus* and BV-associated bacterial communities. Understanding the molecular mechanisms that drive or are induced by BV-associated vaginal microbiota may help identify targets and develop strategies to restore a healthy vaginal state, where would concurrently reduce the risk of STI acquisition. We hypothesised that specific miRNAs are associated with *Lactobacillus*-dominated and BV-associated Community State Types (CST) by affecting specific host functions.

Methods
Leveraging prospectively collected daily vaginal swab samples, the types and abundance of human miRNAs were used to gain insight into host regulatory mechanisms that potentially associate with vaginal microbial community composition shifts using miRNAseq. Random Forest miRNA feature ranking was used to identify miRNAs correlated with types of vaginal microbiota. Additional *in vitro* cell culture experiments were performed to demonstrate the relationship between miRNA expression, vaginal bacterial culture supernatants and epithelial cell proliferation using qPCR and Western blots.

Results
miRNAseq was performed on 100 samples from 16 unique subjects in 3 longitudinal microbiota profile groups. One of the most significant miRNAs associated with BV was miR-193b. *In vitro*, its expression correlated with decreased cell proliferation in cells exposed to *Lactobacillus* spp. culture media relative to *G. vaginalis* culture supernatants.

Conclusion
miR-193b over expression is associated with reduced cell proliferation in non-BV samples. Control of cell proliferation could contribute to reducing the risk of STI in *Lactobacillus* dominated vaginal microbiota.

DEVELOPMENT AND EVALUATION OF IN-HOUSE MULTIPLEX REAL-TIME PCR FOR DETECTION OF NEISSERIA GONORRHOEAE, CHLAMYDIA TRACHOMATIS AND MYCOPLASMA GENITALIUM INFECTION IN INFERTILITY PATIENTS

Introduction
*C. trachomatis*, *N. gonorrhoeae* and *M. genitalium* are important cause of infertility but detection is usually by PCR which has to be performed individually for each pathogen. The aim of this study was to develop *In house* multiplex Real time PCR assay for simultaneous detection of all these pathogens in single run and will also help in detecting co-infection if present thus saving cost and time in cases of infertility.

Methods
The Taqman probe based multiplex qPCR for detection of *C. trachomatis*, *N. gonorrhoeae* and *M. genitalium* was developed using different primers and probes. Analytical sensitivity of multiplex qPCR was determined using pGEMT Easy vector cloned with target genes. The detection limit for each organism was determined using 10 fold dilutions of targets. The multiplex qPCR was evaluated in 248 clinical samples i.e 98 infertile (endometrial biopsy and endocervical swabs) and 150 healthy controls (endocervical swabs). The sensitivity, specificity, positive and negative predictive value (PPV and NPV) of multiplex qPCR was calculated.