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

¹Souza Gf, ²D Ferreira, ³F Cavalcante, ⁴G Souza, ⁵C Hofer, ¹K Santos. ¹Instituto De Microbiologia Paulo De Goes € - UFRJ, Rio De Janeiro – RJ, Brazil; ²Prof. de Microbiologia, Unesa e Uva € - RJ, Rio De Janeiro – RJ, Brazil; ³Prof. Adjunta de Microbiologia – Polo Macaé € - UFRJ, Macaé – RJ, Brazil; ⁴Enfermeiro – UNIABEU, Belford Roxo – RJ, Brazil; ⁵Departamento de Medicina Preventiva, UFRJ, Rio De Janeiro – RJ, Brazil

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Introduction HIV- infected patients are in a high-risk group to develop *Staphylococcus 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 SCC*mec* 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 SCC*mec* IV and the PVL genes were found in 26 isolates of 20 (29.4%) patients. The isolates were included in 11 genotype profiles.

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 nostril 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.

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MICRORNA EXPRESSION INDUCED BY VAGINAL MICROBIOTA CONTROLS CELL PROLIFERATION

¹Steven Smith, ²Mike Humphrys, ²Pawel Gajer, ²Shaikh Iqbal, ¹Vonetta Edwards, ¹Jacques Ravel. ¹University of Maryland, Baltimore, USA; ²Institute For Genome Sciences, Baltimore, USA

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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, which 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.

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

¹Sunil Sethi, ¹Amit Roy, ¹Rakesh Yadev, ¹Rajneesh Dadwal, ²Anuradha Chakraborti, ³Lakhbir Dhaliwal. ¹Department of Medical Microbiology, Pgimer, Chandigarh, India; ²Department of Experimental Medicine and Biotechnology, Pgimer, Chandigarh, India; ³Department of Obstetrics and Gynaecology, Pgimer, Chandigarh, India

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Introduction: C.trachomatis, N.gonorrhoeae and M.genitalium are important cause of infertility but detection is usually by PCR which has to be performed indvidually 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 infecion 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.