FUNGAL MICROBIOME (MYCOBIOME) OF THE UTERINE CERVIX: DOES IT PLAY ANY ROLES IN HUMAN REPRODUCTION?

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INTRODUCTION

Unlike bacterial microbiome, fungal microbiome (mycobiome) of the female reproductive tract is less well-studied. Even less is known about mycobiome of the cervix, which is the last barrier guarding the uterine cavity. We aim to systematically profile fungal taxa in the cervix and explore their roles in pregnancy.

METHODS

Recruitment of participants & sample collection: Women with a singleton pregnancy and a shortened or dilated cervix in the second trimester were recruited for this case-control observational study. A cervical swab sample was collected before pessary ring/cerclage placement. Women were followed-up until one month postdelivery. Spontaneous preterm birth < 37 weeks was regarded as complicated outcome, while term birth as uneventful outcome.

This study was approved by the Institutional Review Boards. Informed consent was obtained from patients attending the Department of Obstetrics and Gynaecology, Prince of Wales Hospital, The Chinese University of Hong Kong or the Department of Obstetrics and Gynaecology, Hallym University, Seoul, South Korea.

Patients were included in this study if they had: (i) shortened (< 25 mm) cervical length or painless cervical dilation (1.5 cm-5.0 cm) in the second trimester; (ii) intact membrane, and (iii) no labour contractions (once per 10 minutes) at the time of study sample collection. However, women were excluded from this study if they had: (i) a multiple pregnancy, (ii) uterine abnormality, or (iii) coitus or vaginal applications 48 hours before sample collection.

Patients were assessed clinically with uterine cervical length measured using transvaginal ultrasound as described by the Fetal Medicine Foundation.[1] Gestational age was assessed by maternal last menstrual period or through first-trimester ultrasound assessments of the crown-rump length.

Before pessary or cerclage intervention, a cervical swab sample was be collected from the patient. All participants were followed up till one month post-delivery to exclude iatrogenic preterm births.

Fungal DNA extraction, broad-range fungal-specific PCR and sequencing: Fungal DNA extracted (YeaStar, Zymo Research) from cervical swabs was PCR-amplified by primers targeting the fungal internal-transcribed spacer 2 (ITS2) DNA and sequenced on the MiSeq platform (Illumina). High-quality reads of >95% nucleotide identity were clustered as an operational taxonomic unit and aligned to the UNITE database[2] by PROTAX, a robust tool for performing statistically reliable identifications of fungi in spite of the incompleteness of extant reference sequence databases and unresolved taxonomic relationships.[3]

Bioinformatics analysis: Mycobiome data comprise many observation of zero counts (i.e. highly-sparsed). Consequently, detecting for changes in mycobiome dataset by T-test, which presumes normal distribution, would be insensitive. Analogous to T-test, investigators have developed differential abundance test based on Zero-Inflated Gaussian (ZIG) model which better describes the highly-sparsed microbiome data. [4] We used the metagenomeSeq R package, which implements ZIG test after normalization to account for the different sequencing depths.[4] To adjust for multiple testing, only taxa with False Discovery Rate-adjusted ZIG *p*-values < 0.05 is considered as differentially abundant.

RESULTS & DISCUSSION

Of 38 women recruited, 13 delivered preterm spontaneously (complicated outcome) and 25 delivered at term (uneventful outcome). In all 38 cervical samples, the most abundant taxa were *Candida*, Agaricomycetes, Agaricales and Ascomycota (**Fig. 1**).

Since *Candida* was the most abundant fungal taxa harboring the cervix of pregnant women, in this poster, we focused on analyzing all *Candida* species detected. There were five *Candida* species in the cervical samples of pregnant women in our study (**Table**). Of these, only two were detected at statistically significantly different abundances between the complicated and uneventful groups. Specifically, *Candida albicans* (3.0-fold; p = 1.3e-15, **Fig. 2**) and *Candida parapsilosis* (9.1-fold; p = 5e-65) were more abundant in the complicated group than the uneventful group.

The data from our cervical mycobiome study on pregnant women echo well with similar studies on vaginal mycobiome on non-pregnant women. Close to 200 fungal taxa, including 16 *Candida* spp., were observed in vaginal tracts of reproductive age women.[5] Moreover, it was reported that fungal diversity was increased in patients with allergic rhinitis and recurrent vaginal candidiasis.[6] The fungal diversity revealed by these mycobiome studies is more than previously recognized.

Table. Differential abundance testing results of all *Candida* species in cervical samples

Taxa	Fold change (SD)	ZIG *p* value	Adjusted ZIG *p* value	
Candida albicans	3.00 ± 1.19	2.9E-17	1.3E-15	*
Candida glabrata	-0.83 ± 1.48	0.08	0.80	
Candida lessepsii	-0.67 ± 5.73	0.48	0.93	
Candida parapsilosis	9.10 ± 1.70	5.6E-67	5.0E-65	*
Candida tropicalis	-1.11 ± 0.97	0.01	0.23	

Notes:
Fold change > 0 represents a higher abundance in the complicated group than the uneventful group.
SD, standard deviation.
ZIG, zero-inflated Gaussian model fit.
1E-23 means 1 x 10^{-23}.

CONCLUSION

Changes in cervical mycobiome is associated with an adverse pregnancy outcome, namely preterm birth. The roles of cervical mycobiome in human reproduction warrant further investigation.

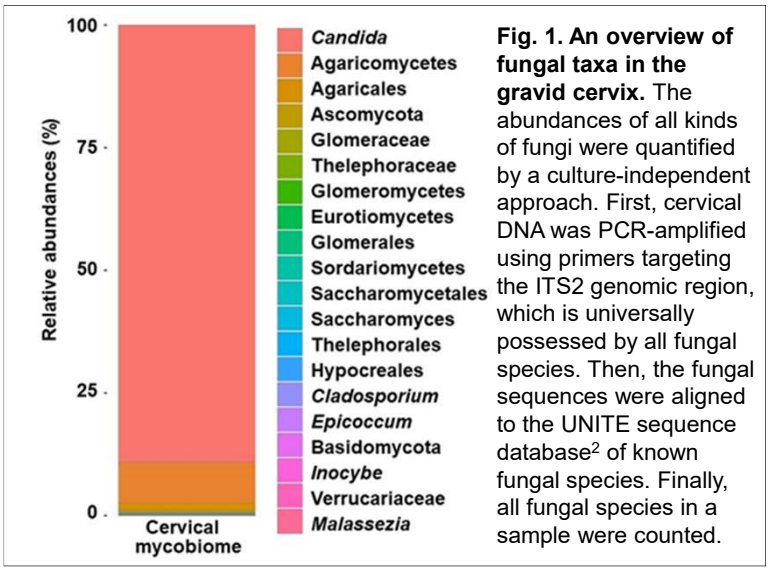

Fig. 1. An overview of fungal taxa in the gravid cervix. The abundances of all kinds of fungi were quantified by a culture-independent approach. First, cervical DNA was PCR-amplified using primers targeting the ITS2 genomic region, which is universally possessed by all fungal species. Then, the fungal sequences were aligned to the UNITE sequence database[2] of known fungal species. Finally, all fungal species in a sample were counted.

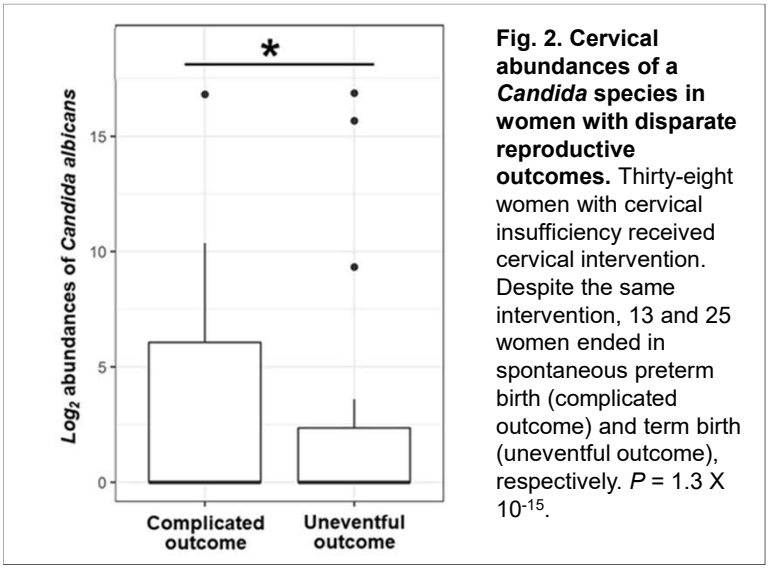

Fig. 2. Cervical abundances of a *Candida* species in women with disparate reproductive outcomes. Thirty-eight women with cervical insufficiency received cervical intervention. Despite the same intervention, 13 and 25 women ended in spontaneous preterm birth (complicated outcome) and term birth (uneventful outcome), respectively. P = 1.3 X 10^{-15}.

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