

SUPPLEMENTARY MATERIAL

Distribution of *ermB*, *ermF*, *tet(W)*, and *tet(M)* resistance genes in the vaginal ecosystem of women during pregnancy and puerperium

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Table S1. Vaginal status, stratified by the gestational age.

Time point	H (n=142)	I (n=51)	BV (n=35)
1 st trimester	33 (23.2%)	26 (51.0%)	13 (37.1%)
2 nd trimester	47 (33.1%)	10 (19.6%)	6 (17.1%)
3 rd trimester	52 (36.6%)	7 (13.7%)	4 (11.4%)
puerperium	10 (7.1%)	8 (15.7%)	12 (34.3%)

Table S2. Prevalence of resistance genes. Prevalence for all cases and those showing a BV vaginal status is shown.

Resistance genes	n° of cases (tot=228)	BV cases (n=35)
None	39 (17.1%)	1 (2.8%)
<i>tet(M)</i>	51 (22.3%)	6 (17.1%)
<i>ermB</i>	10 (4.4%)	1 (2.8%)
<i>ermF</i>	0 (0.0%)	0 (0.0%)
<i>tet(W)</i>	1 (0.4%)	0 (0.0%)
<i>ermB</i> + <i>tet(M)</i>	55 (24.1%)	7 (20.0%)
<i>ermF</i> + <i>tet(M)</i>	8 (3.5%)	2 (5.7%)
<i>tet(M)</i> + <i>tet(W)</i>	1 (0.4%)	0 (0.0%)
<i>ermB</i> + <i>ermF</i>	1 (0.4%)	1 (2.8%)
<i>ermB</i> + <i>tet(W)</i>	0 (0.0%)	0 (0.0%)
<i>ermF</i> + <i>tet(W)</i>	1 (0.4%)	0 (0.0%)
<i>ermB</i> + <i>ermF</i> + <i>tet(M)</i>	31 (13.6%)	10 (28.6%)
<i>ermB</i> + <i>tet(M)</i> + <i>tet(W)</i>	13 (5.7%)	4 (11.4%)
<i>ermB</i> + <i>ermF</i> + <i>tet(W)</i>	1 (0.4%)	0 (0.0%)
<i>ermF</i> + <i>tet(M)</i> + <i>tet(W)</i>	1 (0.4%)	1 (2.8%)
<i>ermB</i> + <i>ermF</i> + <i>tet(M)</i> + <i>tet(W)</i>	15 (6.6%)	2 (5.7%)

Table S3. Distribution of macrolide and tetracycline resistance genes, stratified for the gestational age.

Time point	<i>ermB</i> (n=126)	<i>ermF</i> (n=58)	<i>tet(M)</i> (n=175)	<i>tet(W)</i> (n=33)
1 st trimester	44 (34.9%)	23 (39.6%)	55 (31.4%)	11 (33.4%)
2 nd trimester	33 (26.2%)	11 (19.0%)	46 (26.3%)	8 (24.2%)
3 rd trimester	27 (21.4%)	13 (22.4%)	47 (26.8%)	8 (24.2%)
Puerperium	22 (17.5%)	11 (19.0%)	27 (15.4%)	6 (18.2%)

Table S4. Bacterial genera statistically different ($p<0.05$, Mann-Whitney U-test) between samples. Presence and absence of each resistance gene, stratified according to women's vaginal status, is shown. For each gene and category, the number of samples is indicated.

Gene	Status	N+ ^a	N- ^b	Genus	Avg. rel. ab (%)		Direction ^c
					Gene positive [+]	Gene negative [-]	
<i>ermB</i>	BV	26	9	<i>Prevotella</i>	13.0	2.6	↑
				<i>Megasphaera</i>	7.9	1.3	↑
				<i>Sneathia</i>	5.5	0.0	↑
				<i>Prevotella 7</i>	0.0	3.4	↓
				<i>DNF00809</i>	1.0	0.1	↑
	I	30	21	<i>Prevotella</i>	6.4	2.5	↑
	H	70	72	<i>Prevotella</i>	1.5	0.1	↑
				<i>Atopobium</i>	2.3	0.1	↑
				<i>Streptococcus</i>	0.1	0.3	↓
<i>ermF</i>	BV	16	19	<i>Sneathia</i>	6.2	2.3	↑
				<i>Ureaplasma</i>	1.0	0.1	↑
				<i>Prevotella 6</i>	3.3	0.6	↑
				<i>DNF00809</i>	1.3	0.3	↑
				<i>Fastidiosipila</i>	1.1	0.3	↑
	I	15	36	<i>Lactobacillus</i>	55.5	79.2	↓
				<i>Prevotella</i>	9.0	3.0	↑
				<i>Atopobium</i>	8.1	2.0	↑
				<i>Streptococcus</i>	4.9	0.6	↑
	H	27	115	<i>Lactobacillus</i>	74.3	84.8	↓
				<i>Bifidobacterium</i>	5.2	3.1	↑
				<i>Prevotella</i>	2.8	0.3	↑
				<i>Atopobium</i>	1.3	1.1	↑
<i>tet(M)</i>	BV	32	3	<i>Gardnerella</i>	18.13	76.18	↓
				<i>Dialister</i>	1.45	0.02	↑
				<i>Prevotella 6</i>	2.02	0.00	↑
	I	42	9	--	--	--	--
	H	101	41	<i>Lactobacillus</i>	79.87	90.06	↓
				<i>Bifidobacterium</i>	4.89	0.00	↑
				<i>Prevotella</i>	1.12	0.03	↑
				<i>Atopobium</i>	1.64	0.00	↑
<i>tet(W)</i>	BV	7	28	<i>Megasphaera</i>	13.95	4.30	↑
				<i>Dialister</i>	1.99	1.17	↑
				<i>Prevotella 6</i>	4.51	1.18	↑
				<i>Aerococcus</i>	0.33	1.72	↓
				<i>Fastidiosipila</i>	1.29	0.48	↑
	I	6	45	<i>Lactobacillus</i>	51.74	74.93	↓
				<i>Bifidobacterium</i>	9.91	2.76	↑
	H	20	122	<i>Lactobacillus</i>	72.33	84.53	↓
				<i>Bifidobacterium</i>	4.96	3.24	↑
				<i>Prevotella</i>	3.21	0.41	↑
				<i>Atopobium</i>	0.49	1.28	↓
				<i>Anaerococcus</i>	1.30	0.08	↑

^a Number of samples positive to each resistance gene per each vaginal status

^b Number of samples negative to each resistance gene per each vaginal status

◦ ↑ indicates an increase in gene positive samples with respect to gene negative ones. ↓ indicates the opposite

Table S5. List of primers and PCR conditions used for the detection of resistance genes.

Gene	Primers	PCR conditions	Amplicon size	Reference
<i>ermB</i>	5'-GAAAAGGTACTCAACCAAATA-3' 5'-AGTAACGGTACTTAAATTGTTTAC-3'	95°C for 10 min; 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min; 72°C for 7 min	639 bp	Milanovic, 2017 ^a
<i>ermF</i>	5'-CGGGTCAGCCTTTACTATTG-3' 5'-GGACCTACCTCATAGACAAG-3'	95°C for 10 min; 35 cycles of 94°C for 1 min, 48°C for 1 min and 72°C for 1 min; 72°C for 7 min	466 bp	Sirichoat, 2020 ^b
<i>tet(M)</i>	5'-ACCCGTATACTATTTTCATGCACT-3' 5'-CCTTCCATAACCGCATTTTG-3'	95°C for 10 min; 35 cycles of 94°C for 1 min, 48°C for 1 min and 72°C for 2 min; 72°C for 7 min	1115 bp	Milanovic, 2017 ^a
<i>tet(W)</i>	5'-GAGAGCCTGCTATATGCCAGC-3' 5'-GGGCGTATCCACAATGTTAAC-3'	95°C for 10 min; 35 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 1 min; 72°C for 7 min	168 bp	Milanovic, 2017 ^a
<i>tet(M)-tn916</i>	5'-TACTACCGGTGAACCTGTTTGCCA-3' 5'-TTTAGCCAGCGGTATCAACGAAGC-3'	95°C for 10 min; 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min; 72°C for 7 min	472 bp	Jeters, 2009 ^c

a Milanović V., Osimani A., Aquilanti L., et al. (2017). Occurrence of antibiotic resistance genes in the fecal DNA of healthy omnivores, ovo-lacto vegetarians and vegans. *Mol Nutr Food Res*, 61(9).

b Sirichoat A., Flórez A.B., Vázquez Let al. (2020). Antibiotic susceptibility profiles of lactic acid bacteria from the human vagina and genetic basis of acquired resistances. *Int J Mol Sci*, 21(7):2594

c Jeters R. T., Rivera A. J., Boucek L.M., et al. (2009). Antibiotic resistance genes in the vaginal microbiota of primates not normally exposed to antibiotics. *Microb Drug Resist*, 15(4):309-315

Figure S1. Alpha diversity boxplots for the four resistance genes combination. Boxplot of the alpha-diversities (Faith's phylogenetic diversity metric) of samples grouped according to the combination of four resistance genes tested. Red lines represent median values, whereas blue ones represent means; yellow dots are the values for the single sample in each category; only combinations with >1 sample per group were considered.

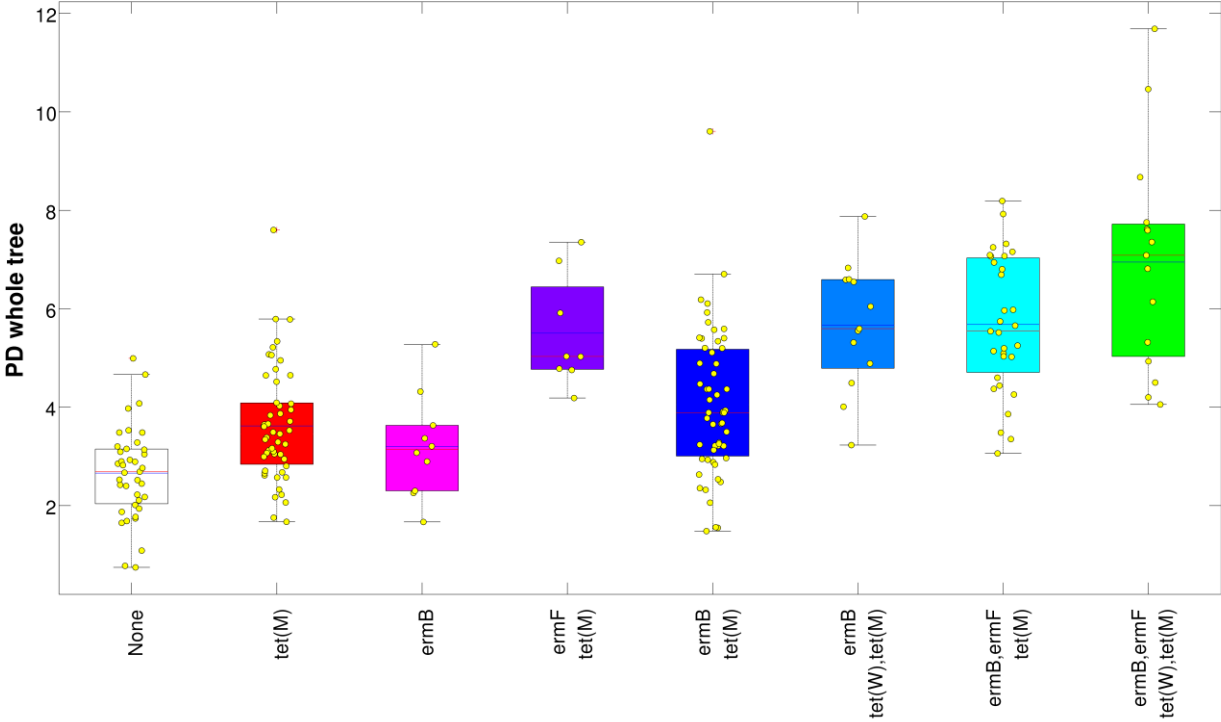


Figure S2. All statistically significant survival curves. For any combination of genus-resistance gene, statistically significant differences (log-rank p -value < 0.05) are shown. For each genus-gene, the lowest abundance threshold for which the test was significant is reported.

