

SUPPLEMENTARY MATERIAL

Linking Antibiotic Resistance Genes (ARGs) in the vaginal microbiota to Health-related Behaviors and Antibiotic Awareness in Reproductive-Age Women: A Cross-Sectional Study

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Figure S1. Principal coordinate analysis (PCoA) plots based on the unweighted UniFrac distance among samples.

Points represent single samples, colored, in each plot, according to presence or absence of the specific ARG; centroids represent the average coordinate of the samples within the same category and ellipses are the SEM-based confidence intervals; for each plot, the first and second coordinate are represented.

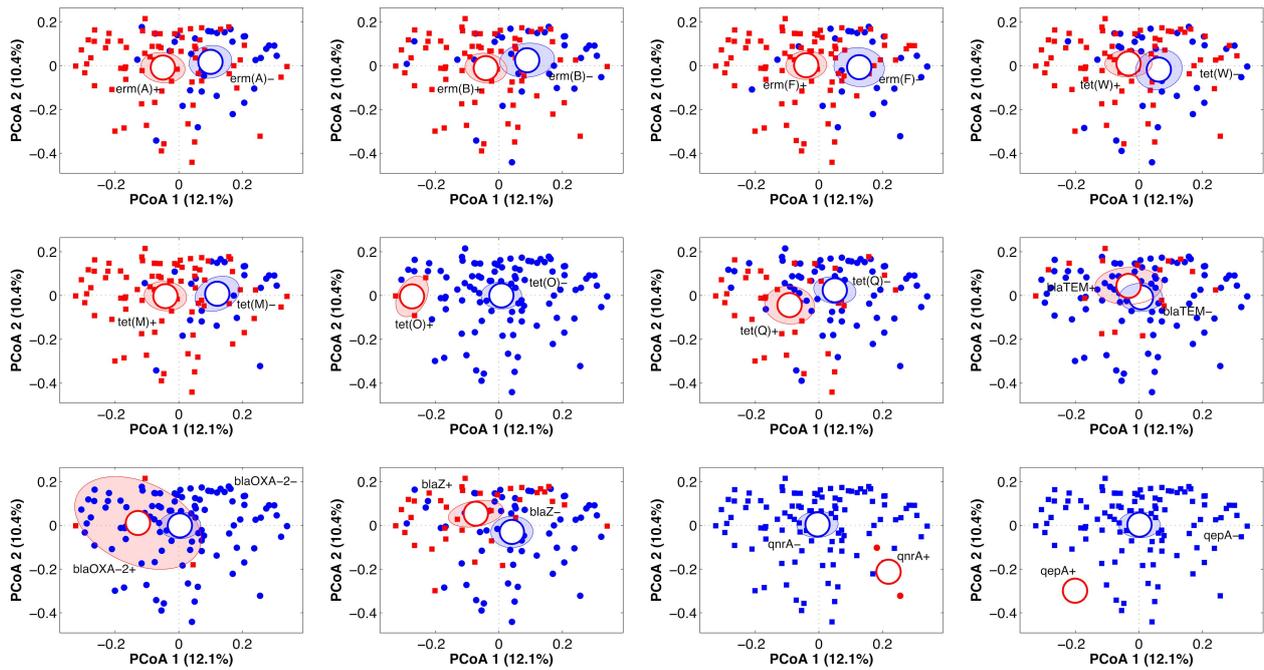


Figure S2. Boxplots of the significantly different taxa over the categorized ARG score. Box and whiskers plot represent the median + IQ range of the data distribution; median is indicated as a red line, whereas the distribution mean is indicated as a blue line. For each taxa, individual abundances are highlighted as gray dots. Statistically different taxa are highlighted by black “*” above the boxplots. “Rel.ab(%)”: relative abundance. For visualization reasons, abundances are represented in log₁₀ scale.

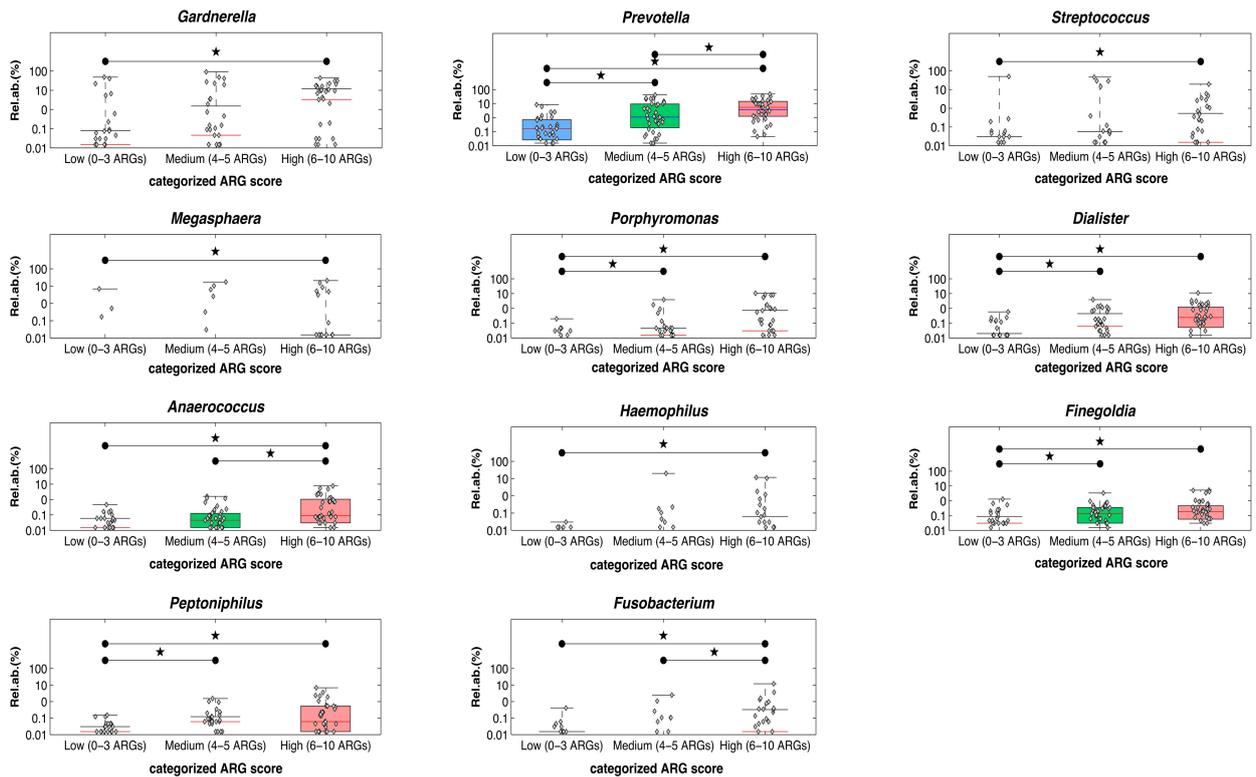


Figure S3. Taxa co-abundance groups (CAGs) analysis plots. (A) Heatmap showing the clusters of co-abundant taxa; heatmap color is proportional to the Spearman's rank correlation among taxa and is clustered according to the Pearson distance and average linkage. Only taxa with an abundance >0.5% in >10% of samples (n=11) over the entire dataset (n=105) were used. The dendrogram on the right highlights the four CAGs obtained. (B) Barplots of the CAGs abundance over the entire dataset. CAGs are named according to the most representative taxon/taxa. The “Other” group comprises all the taxa not used for the CAG heatmap.

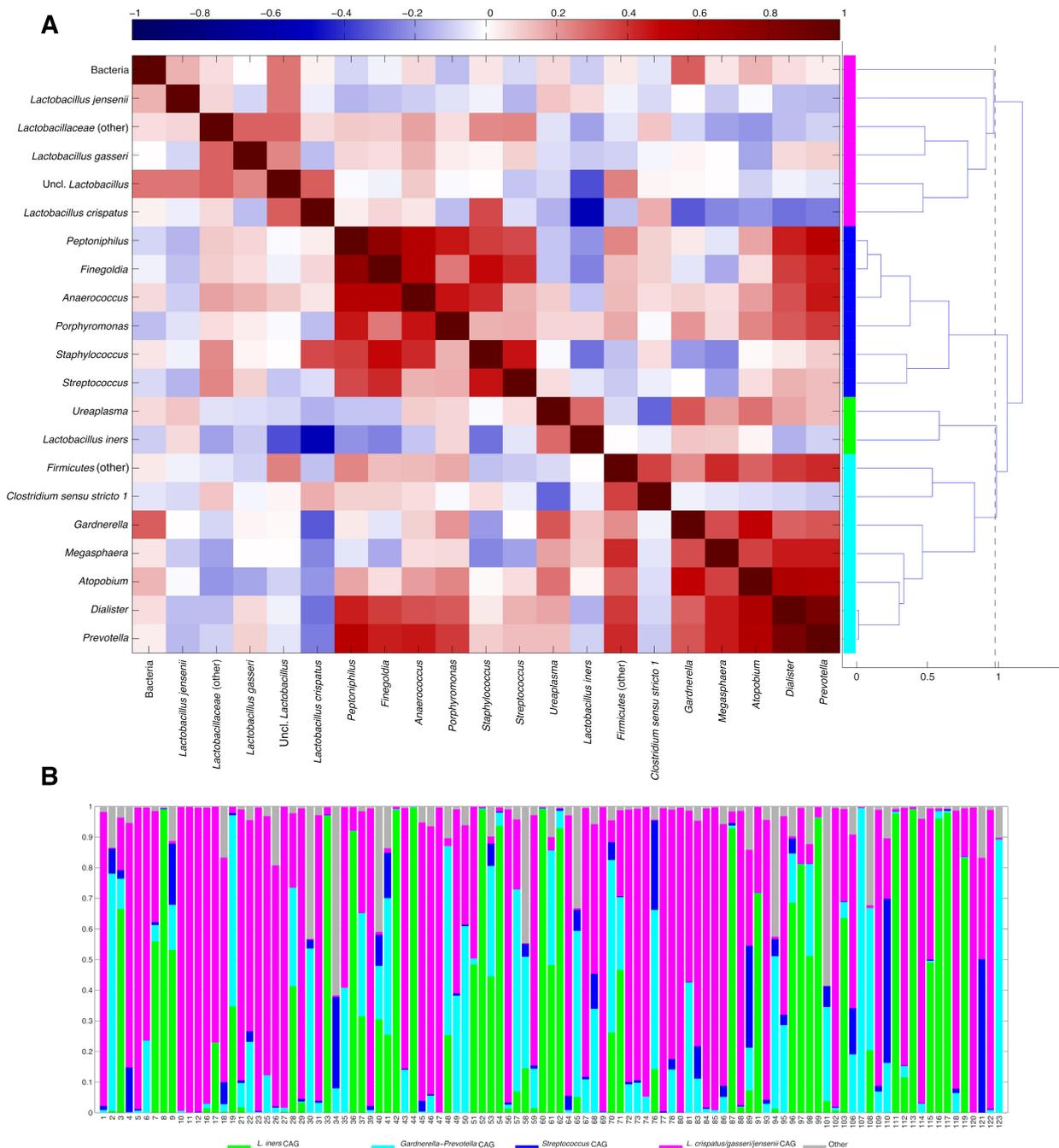


Table S1. List of primer sequences and PCR conditions for the detection of ARGs with modifications. The amplicons were observed on 1%, 1.5% and 2% gel electrophoresis based on their band size.

Gene	Primer sequences	PCR conditions	Amplicon size	References
<i>erm(A)</i>	F 5'-CCCGAAAAATACGCAAATTTTCAT-3' R 5'-CCCTGTTTACCCATTTATAAACG-3'	95°C for 5 min; 30 cycles of 95°C for 1 min, 59°C for 1 min and 72°C for 1 min 30 sec; 72°C for 10 min	590 bp	[1]
<i>erm(B)</i>	F 5'-GAAAAGGTACTCAACCAAATA-3' R 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	[2]
<i>erm(F)</i>	F 5'-CGGGTCAGCACTTTACTATTG-3' R 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	[3, 8]
<i>tet(M)</i>	F 5'-ACCCGTATACTATTTTCATGCACT-3' R 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	[2]
<i>tet(M)-Tn916</i>	F 5'-TACTACCGGTGAACCTGTTTGCCA-3' F 3'-TTAGCCAGCGGTATCAACGAAGC-5'	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	[4]
<i>tet (O)</i>	F 5'-ACGGARAGTTTATTGTATACC-3' R 5'-TGGCGTATCTATAATGTTGAC-3'	94°C for 5 min; 30 cycles of 94°C for 30 sec, 51°C for 30 sec and 72°C and 30 sec; 72°C for 7 min	171 bp	[5]
<i>tet(w)</i>	F 5'-GAGAGCCTGCTATATGCCAGC-3' R 5'-GGGCGTATCCACAATGTTAAC-3'	95°C for 10 min; 35 cycles of 94°C for 1 min, 62°C for 1 min and 72°C per 1 min; 72°C for 7 min	168 bp	[2]
<i>tet(Q)</i>	F 5'-GATACICCIGGICAYRTIGAYTT-3' R 5'-GCCCARWAIGGRTTIGGIGGIACYTC-3'	95°C for 10 min; 35 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 1 min; 72°C for 7 min	904 bp	[2]
<i>tet(Q)-rteA</i>	5'-TGTGGACATTCTCGAACCGATGCT-3' 5'-CTATCTCCTGCCATTCATAGAGGC-3'	95°C for 10 min; 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C and 1 min; 72°C for 7 min	435 bp	[4]
<i>blaOXA-2</i>	F 5'-TTCAAGCCAAAGGCACGATAG-3' R 5'-TCCGAGTTGACTGCCGGGTTG-3'	96°C for 5 min; 35 cycles of 96°C for 1 min, 65°C for 1 min and 72°C for 2 min; 72°C for 10 min	702 bp	[6]
<i>blaTEM</i>	F 5'-TTTCGTGTCGCCCTTATTCC- 3' R 5'-CCGGCTCCAGATTTATCAGC-3'	94°C for 5 min; 30 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 1 min; 72°C for 5 min	692 bp	[7]
<i>blaZ</i>	F 5'-ACTTCAACACCTGCTGCTTTC-3' R 5'-TGACCACTTTTATCAGCAA-3'	95°C for 5 min; 30 cycles of 95°C for 1 min, 59°C for 1 min and 72°C for 1 min 30 sec; 72°C for 10 min	173 bp	[1]
<i>blaSHV</i>	F 5'-TCGCCTGTGTATTATCTCCC-3' R 5'-CGCAGATAAATCACCACAATG-3'	94 °C for 5 min; 30 cycles of 94 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 90 sec; 72 °C for 10 min	768 bp	[1]
<i>blaCTX-M</i>	F 5'-TTTGCGATGTGCAGTACCAGTAA-3' R 5'-CGATATCGTTGGTGGTGCCATA-3'	94 °C for 5 min; 30 cycles of 94 °C for 30 sec, 56 °C for 1 min and 72 °C for 60 sec; 72 °C for 10 min	543 bp	[1]
<i>qnrA</i>	F 5'-ATTTCTCACGCCAGGATTTG-3' R 5'-GATCGGCAAAGGTTAGGTCA-3'	94 °C for 2 min; 35 cycles of 94 °C for 30 sec, 53 °C for 30 sec and 72 °C for 60 sec; 72 °C for 10 min	516 bp	[1]
<i>qepA</i>	F 5'-CTTCTCTGGATCCTGGACAT-3'	94 °C for 2 min; 35 cycles of 94 °C for 30 sec, 54 °C	720 bp	[1]

R 5'-TGAAGATGTAGACGCCGAAC-3'

for 30 sec and 72 °C for 60 sec; 72 °C for 10 min

References

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Table S2. List of taxa in each co-abundant group (CAG). CAGs are named according to the most representative taxon/taxa. See Figure S3 for more details.

CAG	Taxa
<i>L. iners</i> CAG	<i>Lactobacillus iners</i> <i>Ureaplasma</i>
<i>Gardnerella-Prevotella</i> CAG	<i>Gardnerella</i> <i>Prevotella</i> <i>Atopobium</i> <i>Megasphaera</i> <i>Dialister</i> <i>Clostridium sensu stricto 1</i> <i>Other Firmicutes</i>
<i>L. crispatus/gasseri/jensenii</i> CAG	<i>Lactobacillus crispatus</i> <i>Lactobacillus gasseri</i> <i>Lactobacillus jensenii</i> Unclassified <i>Lactobacillus</i> <i>Other Bacteria</i> <i>Other Lactobacillaceae</i>
<i>Streptococcus</i> CAG	<i>Streptococcus</i> <i>Porphyromonas</i> <i>Anaerococcus</i> <i>Fingoldia</i> <i>Peptoniphilus</i> <i>Staphylococcus</i>