Interpretation Guide for Microbiome Analysis Script

This document explains how to interpret each of the outputs generated by the analysis script. It is structured to follow the same flow as the script: descriptive stats → plots → models → hypothesis tests → regression.

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1. Descriptive Statistics

- **Shannon diversity (D1 vs D6):**
- Higher Shannon at D6 compared to D1 → recovery/expansion of microbial diversity after antibiotics.
- Lower Shannon at D6 → suppression of microbial diversity due to antibiotics.

- Stable Shannon → resilience of the microbiome.

- **Chao1 richness (D1 vs D6):**

- Lower Chao1 at D6 → reduced species richness, antibiotics eliminate rarer taxa.

- Higher/stable Chao1 → resilience or rapid recolonization of taxa.

- **Jaccard distance (D1 vs D6):**

- High values → strong reshuffling of microbial composition between D1 and D6.
- Low values → microbiome composition remains relatively stable.

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2. Correlation Analysis

- **Correlation between D1 and D6 diversity:**
- Strong positive correlation → patients with high baseline diversity remain relatively high post-treatment.
- Weak/no correlation → antibiotics override baseline differences, leveling patients to a similar diversity.

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3. Plots

- **Histograms of Shannon/Chao1:**
- Shape shows distribution across patients.
- Right-skewed: most patients have low diversity, few with very high diversity.

- Normal/bell-shaped: diversity evenly distributed.

- **Bar chart of diversity by Antibiotic class:**

- Shows average diversity change across drug classes.

- Differences across bars suggest some antibiotics have harsher impacts.

- **Boxplots of diversity by Antibiotic class:**

- Median line = central tendency of diversity.
- Whiskers = variability.

- Outliers = individuals with unusual responses (clinically interesting).

- **Scatterplot of D1 vs D6 with regression line:**

- Slope close to 1 → diversity largely preserved.
- Slope < 1 → diversity declines.
- Different slopes by antibiotic class → drug-dependent impacts.

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4. Outlier Detection

- Outliers are patients with unexpected diversity values (extreme drop or increase).
- Important to note biologically: may indicate antibiotic sensitivity or resistance.
- Should not be blindly removed—could hold clinically relevant patterns.

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5. Assumption Checks

- **Normality (residual plots, Shapiro test):**

- If residuals are non-normal → p-values less reliable; consider transformations or nonparametric models.

- **Homoscedasticity (equal variance across groups):**

- Equal variance → model assumptions valid.
- Unequal variance → prefer Welch's test or heteroscedastic regression.

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6. Confidence Intervals

- **Narrow intervals** → consistent patient responses.
- **Wide intervals** → high variability across individuals.

• As confidence level increases (90% → 95% → 99%), intervals widen to account for more uncertainty.
• Useful for reporting *certainty ranges* rather than point estimates.

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7. Hypothesis Tests

• **Male vs Female (placebo):**
• If no difference → sex not a major factor in microbiome stability without antibiotics.

• If difference → biological sex may influence baseline diversity.

• **Dose comparisons (10 vs 0):**

• If significant → highest dose reduces diversity compared to placebo.

• If not significant → dose may not strongly impact diversity at that comparison.

• **Across all doses (ANOVA + post-hoc):**

• Significant ANOVA → at least one dose differs.
• Post-hoc (e.g., Tukey HSD) shows *which* doses differ.

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8. Linear Regression Results

• **Coefficients:**
• Positive coefficient for baseline diversity → higher baseline predicts higher diversity post-antibiotic.

• Antibiotic class coefficients → effect relative to baseline class (e.g., OBL).

• **Confidence intervals for coefficients:**

• CI excluding 0 → reliable effect.

• CI including 0 → effect uncertain.

• **Model fit (R^2, residuals):**

• High R^2 → model explains much of the variation.

• Low R^2 → other unmeasured factors may be driving diversity changes.

• **Predictions (example: dose = 3 mg/day):**

• Allows practical interpretation for clinical settings.
• If predicted diversity is much lower than placebo → clear evidence of dose impact.

9. Key Takeaways for Biological Interpretation

- Antibiotics can reduce both diversity and richness, but the extent depends on class and dose.
- Baseline diversity may provide resilience—patients with richer microbiomes resist perturbation.
- Some antibiotics cause major reshuffling (high Jaccard), others less so.
- Individual variability is substantial: not all patients respond the same.

This file should be read **alongside the script output**. After running each block, check here for what the statistical or visual result means biologically and clinically.