

*Supplementary Materials*

# Antimicrobial Resistance Profiles, Virulence Determinants, and Biofilm Formation in Enterococci Isolated from Rhesus Macaques (*Macaca mulatta*): A Potential Threat for Wildlife in Bangladesh?

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## Supplementary Materials

**Supplementary Table S1.** Pearson correlation coefficient to assess the pairs of any of two virulence genes detected in *E. faecalis* isolated from rectal swab of samples of rhesus macaques.

|             |                     | agg    | fsrA    | fsrB    | fsrC   | pil     | gelE   | sprE  | ace | cyl |
|-------------|---------------------|--------|---------|---------|--------|---------|--------|-------|-----|-----|
| <i>agg</i>  | Pearson Correlation | 1      |         |         |        |         |        |       |     |     |
|             | Sig. (2-tailed)     |        |         |         |        |         |        |       |     |     |
| <i>fsrA</i> | Pearson Correlation | 0.142  | 1       |         |        |         |        |       |     |     |
|             | Sig. (2-tailed)     | 0.358  |         |         |        |         |        |       |     |     |
| <i>fsrB</i> | Pearson Correlation | -0.069 | 0.017   | 1       |        |         |        |       |     |     |
|             | Sig. (2-tailed)     | 0.656  | 0.912   |         |        |         |        |       |     |     |
| <i>fsrC</i> | Pearson Correlation | -0.048 | -0.118  | -0.069  | 1      |         |        |       |     |     |
|             | Sig. (2-tailed)     | 0.759  | 0.444   | 0.656   |        |         |        |       |     |     |
| <i>pil</i>  | Pearson Correlation | -0.048 | 0.402** | -0.069  | -0.048 | 1       |        |       |     |     |
|             | Sig. (2-tailed)     | 0.759  | 0.007   | 0.656   | 0.759  |         |        |       |     |     |
| <i>gelE</i> | Pearson Correlation | -0.059 | 0.068   | -0.086  | -0.059 | -0.059  | 1      |       |     |     |
|             | Sig. (2-tailed)     | 0.703  | 0.659   | 0.581   | 0.703  | 0.703   |        |       |     |     |
| <i>sprE</i> | Pearson Correlation | -0.033 | 0.281   | 0.482** | -0.033 | -0.033  | -0.041 | 1     |     |     |
|             | Sig. (2-tailed)     | 0.83   | 0.064   | 0.001   | 0.83   | 0.83    | 0.79   |       |     |     |
| <i>ace</i>  | Pearson Correlation | 0.142  | 0.871** | 0.017   | -0.118 | 0.402** | -0.147 | 0.281 | 1   |     |
|             | Sig. (2-tailed)     | 0.358  | 0       | 0.912   | 0.444  | 0.007   | 0.342  | 0.064 |     | .   |
| <i>cyl</i>  | Pearson Correlation | .a     | .a      | .a      | .a     | .a      | .a     | .a    | .a  | .a  |
|             | Sig. (2-tailed)     | .      | .       | .       | .      | .       | .      | .     | .   | .   |

\*\*Correlation is significant at the 0.01 level (2-tailed). <sup>a</sup>Can-t be computed because at least one of the variables is constant.

**Supplementary Table S2.** Association in the detection of virulence genes and determination of biofilm formation in *E. faecalis* (n = 44) isolated from rectal swab samples of rhesus macaques.

| Virulence genes | Virulence in different degrees of biofilm formation |  |                                    | <i>p</i> -value |
|-----------------|---|--|------------------------------------|-----------------|
|                 | No. (%) strong biofilm former (n = 9)               | No. (%) intermediate biofilm former (n = 26) | No. (%) non-biofilm former (n = 9) |                 |
| <i>agg</i>      | 9 (100 <sup>a,b</sup> )                             | 26 (100 <sup>b</sup> )                       | 7 (77.8 <sup>a</sup> )             | 0.017           |
| <i>fsrA</i>     | 9 (100 <sup>a</sup> )                               | 26 (100 <sup>a</sup> )                       | 0 (0 <sup>b</sup> )                | <0.001          |
| <i>fsrB</i>     | 9 (100 <sup>a</sup> )                               | 23 (88.5 <sup>a</sup> )                      | 9 (100 <sup>a</sup> )              | 0.328           |
| <i>fsrC</i>     | 9 (100 <sup>a</sup> )                               | 25 (96.2 <sup>a</sup> )                      | 9 (100 <sup>a</sup> )              | 0.702           |
| <i>pil</i>      | 9 (100 <sup>a,b</sup> )                             | 26 (100 <sup>b</sup> )                       | 7 (77.8 <sup>a</sup> )             | 0.017           |
| <i>gelE</i>     | 9 (100 <sup>a</sup> )                               | 23 (88.5 <sup>a</sup> )                      | 9 (100 <sup>a</sup> )              | 0.328           |
| <i>sprE</i>     | 9 (100 <sup>a</sup> )                               | 26 (100 <sup>a</sup> )                       | 8 (88.9 <sup>a</sup> )             | 0.137           |
| <i>ace</i>      | 9 (100 <sup>a</sup> )                               | 26 (100 <sup>a</sup> )                       | 0 (0 <sup>b</sup> )                | <0.001          |
| <i>cyl</i>      | 0 (0 <sup>a</sup> )                                 | 0 (0 <sup>a</sup> )                          | 0 (0 <sup>a</sup> )                | NA              |

Here, values with different superscripts differ significantly (P < 0.05) within the variable under assessment, CI= Confidence interval, NA= Not applied.

**Supplementary Table S3.** Association of antibiotic resistance patterns and biofilm formation in *E. faecalis* strains detected in rectal swab samples of rhesus macaques.

| Categories | Antibiotics   | Antibiotic resistance in different degrees of biofilm formation |  |                                    | <i>p</i> -value |
|------------|---------------|---|--|------------------------------------|-----------------|
|            |               | No. (%) strong biofilm former (n = 9)                           | No. (%) intermediate biofilm former (n = 26) | No. (%) non-biofilm former (n = 9) |                 |
| Phenotypic | CIP           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                          | 0 (0 <sup>a</sup> )                | NA              |
|            | TE            | 2 (22.2 <sup>a</sup> )  | 6 (23.1 <sup>a</sup> )                       | 1 (11.1 <sup>a</sup> )             | 0.737           |
|            | LEV           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                          | 0 (0 <sup>a</sup> )                | NA              |
|            | FOS           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                          | 0 (0 <sup>a</sup> )                | NA              |
|            | RD            | 9 (100 <sup>a</sup> )   | 26 (100 <sup>a</sup> )                       | 9 (100 <sup>a</sup> )              | NA              |
|            | P             | 9 (100 <sup>a</sup> )   | 26 (100 <sup>a</sup> )                       | 9 (100 <sup>a</sup> )              | NA              |
|            | LZD           | 9 (100 <sup>a</sup> )   | 16 (61.5 <sup>a</sup> )                      | 1 (11.1 <sup>b</sup> )             | 0.001           |
|            | NOR           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                          | 0 (0 <sup>a</sup> )                | NA              |
|            | NIT           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                          | 0 (0 <sup>a</sup> )                | NA              |
|            | AMP           | 9 (100 <sup>a</sup> )   | 26 (100 <sup>a</sup> )                       | 9 (100 <sup>a</sup> )              | NA              |
|            | C             | 2 (22.2 <sup>a</sup> )  | 1 (3.8 <sup>a</sup> )                        | 0 (0 <sup>a</sup> )                | 0.112           |
|            | VA            | 9 (100 <sup>a</sup> )   | 13 (50 <sup>b</sup> )                        | 1 (11.1 <sup>b</sup> )             | 0.001           |
| Genotypic  | E             | 9 (100 <sup>a</sup> )   | 15 (57.7 <sup>a,b</sup> )                    | 2 (22.2 <sup>b</sup> )             | 0.003           |
|            | <i>blaTEM</i> | 9 (100 <sup>a</sup> )   | 12 (46.2 <sup>b</sup> )                      | 6 (66.7 <sup>a,b</sup> )           | 0.016           |

Here, values with different superscripts differ significantly (P < 0.05) within the variable under assessment, CI= Confidence interval, NA= Not applied.

**Supplementary Table S4:** Pearson correlation coefficient to assess the pairs of any of two virulence genes detected in *E. faecium* isolated from rectal swab samples of rhesus macaques

| Correlations |                     |         |         |         |         |        |         |         |         |     |
|--------------|---------------------|---------|---------|---------|---------|--------|---------|---------|---------|-----|
|              |                     | agg     | fsrA    | fsrB    | fsrC    | pil    | gelE    | sprE    | ace     | cyl |
| agg          | Pearson Correlation | 1       |         |         |         |        |         |         |         |     |
|              | Sig. (2-tailed)     |         |         |         |         |        |         |         |         |     |
| fsrA         | Pearson Correlation | 0.48    | 1       |         |         |        |         |         |         |     |
|              | Sig. (2-tailed)     | 0.07    |         |         |         |        |         |         |         |     |
| fsrB         | Pearson Correlation | 0.48    | 0.722** | 1       |         |        |         |         |         |     |
|              | Sig. (2-tailed)     | 0.07    | 0.002   |         |         |        |         |         |         |     |
| fsrC         | Pearson Correlation | 0.48    | 0.722** | 0.722** | 1       |        |         |         |         |     |
|              | Sig. (2-tailed)     | 0.07    | 0.002   | 0.002   |         |        |         |         |         |     |
| pil          | Pearson Correlation | 0.207   | -0.185  | -0.185  | -0.185  | 1      |         |         |         |     |
|              | Sig. (2-tailed)     | 0.459   | 0.51    | 0.51    | 0.51    |        |         |         |         |     |
| gelE         | Pearson Correlation | 0.48    | 0.722** | 0.722** | 0.722** | -0.185 | 1       |         |         |     |
|              | Sig. (2-tailed)     | 0.07    | 0.002   | 0.002   | 0.002   | 0.51   |         |         |         |     |
| sprE         | Pearson Correlation | 0.48    | 0.444   | 0.444   | 0.444   | 0.431  | 0.444   | 1       |         |     |
|              | Sig. (2-tailed)     | 0.07    | 0.097   | 0.097   | 0.097   | 0.109  | 0.097   |         |         |     |
| ace          | Pearson Correlation | 0.650** | 0.739** | 0.739** | 0.739** | -0.023 | 0.739** | 0.739** | 1       |     |
|              | Sig. (2-tailed)     | 0.009   | 0.002   | 0.002   | 0.002   | 0.936  | 0.002   | 0.002   |         |     |
| cyl          | Pearson Correlation | 0.423   | 0.48    | 0.48    | 0.48    | -0.237 | 0.48    | 0.48    | 0.650** | 1   |
|              | Sig. (2-tailed)     | 0.116   | 0.07    | 0.07    | 0.07    | 0.396  | 0.07    | 0.07    | 0.009   |     |

\*\*Correlation is significant at the 0.01 level (2-tailed).

**Supplementary Table S05.** Association in the detection of virulence genes and determination of biofilm formation in *E. faecium* (n = 15) isolated from rectal swab samples of rhesus macaques.

| Virulence genes | Virulence in different degrees of biofilm formation |   |                                    | p-value |
|-----------------|---|---|------------------------------------|---------|
|                 | No. (%) strong biofilm former (n = 3)               | No. (%) intermediate biofilm former (n = 9) | No. (%) non-biofilm former (n = 3) |         |
| agg             | 2 (66.7 <sup>a</sup> )                              | 0 (0 <sup>b</sup> )                         | 0 (0 <sup>a,b</sup> )              | 0.010   |
| fsrA            | 3 (100 <sup>a</sup> )                               | 3 (33.3 <sup>a,b</sup> )                    | 0 (0 <sup>b</sup> )                | 0.036   |
| fsrB            | 3 (100 <sup>a</sup> )                               | 3 (33.3 <sup>a,b</sup> )                    | 0 (0 <sup>b</sup> )                | 0.036   |
| fsrC            | 3 (100 <sup>a</sup> )                               | 3 (33.3 <sup>a,b</sup> )                    | 0 (0 <sup>b</sup> )                | 0.036   |
| pil             | 1 (33.3 <sup>a</sup> )                              | 3 (33.3 <sup>a</sup> )                      | 0 (0 <sup>a</sup> )                | 0.506   |
| gelE            | 3 (100 <sup>a</sup> )                               | 3 (33.3 <sup>a,b</sup> )                    | 0 (0 <sup>b</sup> )                | 0.036   |
| sprE            | 3 (100 <sup>a</sup> )                               | 3 (33.3 <sup>a,b</sup> )                    | 0 (0 <sup>b</sup> )                | 0.036   |
| ace             | 3 (100 <sup>a</sup> )                               | 1 (11.1 <sup>b</sup> )                      | 0 (0 <sup>b</sup> )                | 0.005   |
| cyl             | 0 (0 <sup>a</sup> )                                 | 0 (0 <sup>b</sup> )                         | 0 (0 <sup>a</sup> )                | NA      |

Here, values with different superscripts differ significantly (P < 0.05) within the variable under assessment, CI= Confidence interval, NA= Not applied.

**Supplementary Table S6.** Association of antibiotic resistance patterns and biofilm formation in *E. faecium* strains detected in rectal swab samples of rhesus macaques.

| Categories | Antibiotics   | Antibiotic resistance in different degrees of biofilm formation |   |                                    | <i>p</i> -value |
|------------|---------------|---|---|------------------------------------|-----------------|
|            |               | No. (%) strong biofilm former (n = 3)                           | No. (%) intermediate biofilm former (n = 9) | No. (%) non-biofilm former (n = 3) |                 |
| Phenotypic | CIP           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                         | 0 (0 <sup>a</sup> )                | NA              |
|            | TE            | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                         | 0 (0 <sup>a</sup> )                | NA              |
|            | LEV           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                         | 0 (0 <sup>a</sup> )                | NA              |
|            | FOS           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                         | 0 (0 <sup>a</sup> )                | NA              |
|            | RD            | 3 (100 <sup>a</sup> )   | 9 (100 <sup>a</sup> )                       | 3 (100 <sup>a</sup> )              | NA              |
|            | P             | 3 (100 <sup>a</sup> )   | 9 (100 <sup>a</sup> )                       | 3 (100 <sup>a</sup> )              | NA              |
|            | LZD           | 3 (100 <sup>a</sup> )   | 6 (66.7 <sup>a,b</sup> )                    | 0 (0 <sup>b</sup> )                | 0.036           |
|            | NOR           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                         | 0 (0 <sup>a</sup> )                | NA              |
|            | NIT           | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                         | 0 (0 <sup>a</sup> )                | NA              |
|            | AMP           | 3 (100 <sup>a</sup> )   | 9 (100 <sup>a</sup> )                       | 3 (100 <sup>a</sup> )              | NA              |
|            | C             | 0 (0 <sup>a</sup> )   | 0 (0 <sup>a</sup> )                         | 0 (0 <sup>a</sup> )                | NA              |
|            | VA            | 3 (100 <sup>a</sup> )   | 4 (44.4 <sup>a,b</sup> )                    | 0 (0 <sup>b</sup> )                | 0.048           |
|            | E             | 3 (100 <sup>a</sup> )   | 9 (100 <sup>a</sup> )                       | 3 (100 <sup>a</sup> )              | NA              |
| Genotypic  | <i>blatem</i> | 3 (100 <sup>a</sup> )   | 6 (66.7 <sup>a,b</sup> )                    | 0 (0 <sup>b</sup> )                | 0.036           |

Here, values with different superscripts differ significantly ( $P < 0.05$ ) within the variable under assessment, CI = Confidence interval, NA= Not applied.