

SUPPLEMENTARY DATA

**RESPIRATORY AND ENTERIC BACTERIAL PATHOGENS IN MUNICIPAL WASTEWATER: A
POTENTIAL RISK OF INFECTION TO WORKERS**

Evida Poopedi^{1,2, 3*}, Tanusha Singh^{2,4} and Annancietar Gomba²

Supplementary Table S1: Gene fragments used to construct a synthetic oligonucleotide gBlocks for PCR standards

| Organisms | Target gene | Amplicon size (bp) | Amplicon sequence |
|----------------------------|-------------|--------------------|---|
| <i>Legionella</i> species | <i>ssrA</i> | 101 | TTTCATCGTTTGCATTTATATTTATTGAGTCTGATTTACGAGA GTTCTCATTCTCGGCATGCACCTCTGGTTTTGCAACCCACGTC GAAGCCAGGTCGCC |
| <i>L. pneumophila</i> | <i>mip</i> | 115 | TTGTCTTATAGCATTGGTGCCGATTTGGGGAAGAATTTTAAAA ATCAAGGCATAGATGTTAATCCGGAAGCAATGGCTAAAGGC ATGCAAGACGCTATGAGTGGCGCTCAATTGG |
| <i>L. pneumophila</i> sg 1 | <i>wzm</i> | 70 | TGCCTCTGGCTTTCAGATTATTTTATTACTCCACTCCAGCGATT TACCCTGTTTCTGCTGTGCCTGTGTG |
| <i>A. hydrophila</i> | <i>aer</i> | 309 | CAAGAACAAGTTCAAGTGGCCACTGGTGGGGGAAACCCAAC TCTCCATCGAGATTTCGTGCCAATCAGTCCTGGGCGTCCCAGA ACGGGGGCTCGACCACCACCTCCCTGTCTCAGTCCGTGCGAC CGACTGTGCCGGCCCGCTCCAAGATCCCGGTGAAGATAGAGC TCTACAAGGCCGACATCTCCTATCCCTATGAGTTCAAGGCCG ATGTCAGCTATGACCTGACCCTGAGCGGCTTCTGCGCTGGG GCGGCAACGCCTGGTATACCCACCCGGACAACCGTCCGAAC GGAACCACACCTTCGT |
| <i>A. butzleri</i> | 16S rDNA | 401 | CCTGGACTTGACATAGTAAGAATGATTTAGAGATAGATTAGT GTCTGCTTGCAGAACTTGCATACAGGTGCTGCACGGCTGTC GTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGA GCGCAACCCTCGTCCTTAGTTGCTAACAGTTCGGCTGAGA CTAAGGAGACTGCCTACGCAAGTAGGAGGAAGGTGAGGATG ACGTCAAGTCATCATGGCCCTTACGTCCAGGGCTACACACGT GCTACAATGGGGTATACAAAGAGCAGCAATACGGTGACGTG GAGCAAATCTCAAAAATGCCTCCCAGTTTCGGATTGTAGTCTG CAACTCGACTACATGAAGTTGGAATCGCTAGTAATCGTAGAT CAGCTATGCTACGGTGAATACG |

Supplementary Table S2: Amplification efficiency, correlation coefficient, slope and coefficient of variance (CV) of the standard curves for qPCR.

| Organism | Amplification efficiency (%) | Correlation coefficient (r2) | Slope | Mean of CV | ±SD |
|---|------------------------------|------------------------------|-------|------------|-----|
| Multiplex assay 1 (<i>Legionella</i> spp., <i>L. pneumophila</i> and <i>L. pneumophila</i> sg 1) | 101 | 0.99 | -3.3 | 0.65 ± 0.7 | |
| | 98 | 0.99 | -3.4 | 0.27 ± 0.1 | |
| | 98 | 0.99 | -3.4 | 0.25 ± 0.2 | |
| <i>Legionella</i> spp. singleplex | 100 | 0.99 | -3.3 | 0.45 ± 0.4 | |
| <i>L. pneumophila</i> sg 1 singleplex | 94 | 1 | -3.5 | 0.38 ± 0.3 | |
| <i>L. pneumophila</i> sg 1 singleplex | 92 | 0.99 | -3.5 | 0.74 ± 0.6 | |
| Multiplex assay 2 (<i>A. hydrophila</i> and <i>A. butzleri</i>) | 91 | 0.99 | -3.6 | 0.45 ± 0.4 | |
| | 93 | 0.99 | -3.5 | 1.03 ± 0.9 | |
| <i>A. hydrophila</i> singleplex | 90 | 0.99 | -3.6 | 0.82 ± 1.4 | |
| <i>A. butzleri</i> singleplex | 93 | 1 | -3.5 | 0.39 ± 0.3 | |

Supplementary Table S3: Comparison of viable *L. pneumophila* across all plants

| WWTP | P value | | |
|------------|----------|------------------|--------------|
| | Influent | Activated sludge | SST effluent |
| 2 versus 1 | 1.000 | 1.000 | 1.000 |
| 3 versus 1 | 0.247 | 0.778 | 1.000 |
| 4 versus 1 | 0.000* | 1.000 | 1.000 |
| 5 versus 1 | 0.004* | 0.975 | 1.000 |
| 3 versus 2 | 1.000 | 0.042* | 1.000 |
| 4 versus 2 | 0.049* | 1.000 | 1.000 |
| 5 versus 2 | 0.284 | 0.066 | 1.000 |
| 4 versus 3 | 0.355 | 0.678 | 1.000 |
| 5 versus 3 | 1.000 | 1.000 | 1.000 |
| 5 versus 4 | 1.000 | 0.844 | 1.000 |

* ($p < 0.05$)**Supplementary Table S4:** Comparison of *Legionella* spp. gene copies across all plants

| WWTP | P value | | |
|------------|----------|------------------|--------------|
| | Influent | Activated sludge | SST effluent |
| 2 versus 1 | 1.000 | 0.179 | 1.000 |
| 3 versus 1 | 1.000 | 1.000 | 1.000 |
| 4 versus 1 | 1.000 | 1.000 | 1.000 |
| 5 versus 1 | 1.000 | 0.347 | 1.000 |
| 3 versus 2 | 1.000 | 0.977 | 1.000 |
| 4 versus 2 | 1.000 | 1.000 | 1.000 |
| 5 versus 2 | 1.000 | 1.000 | 1.000 |
| 4 versus 3 | 1.000 | 1.000 | 1.000 |
| 5 versus 3 | 1.000 | 1.000 | 1.000 |
| 5 versus 4 | 1.000 | 1.000 | 1.000 |

Supplementary Table S5: Comparison of *L. pneumophila* gene copies across all plants

| WWTP | P value | | |
|------------|----------|------------------|--------------|
| | Influent | Activated sludge | SST effluent |
| 2 versus 1 | 0.895 | 1.000 | 0.277 |
| 3 versus 1 | 1.000 | 1.000 | 1.000 |
| 4 versus 1 | 0.099 | 0.035* | 0.002* |
| 5 versus 1 | 0.135 | 1.000 | 1.000 |
| 3 versus 2 | 1.000 | 1.000 | 0.005* |
| 4 versus 2 | 1.000 | 0.130 | <0.001* |
| 5 versus 2 | 1.000 | 1.000 | 0.071 |
| 4 versus 3 | 1.000 | 1.000 | 0.151 |
| 5 versus 3 | 1.000 | 1.000 | 1.000 |
| 5 versus 4 | 1.000 | 0.229 | 0.011* |

* ($p < 0.05$)

Supplementary Table S6: Comparison of *L. pneumophila* serogroup 1 gene copies across all plants

| WWTP | <i>P</i> value | | |
|------------|----------------|------------------|--------------|
| | Influent | Activated sludge | SST effluent |
| 2 versus 1 | 1.000 | 1.000 | 1.000 |
| 3 versus 1 | 1.000 | 1.000 | 0.756 |
| 4 versus 1 | 0.575 | 0.009* | <0.001* |
| 5 versus 1 | 0.395 | 1.000 | 0.223 |
| 3 versus 2 | 1.000 | 1.000 | 0.057 |
| 4 versus 2 | 1.000 | 0.086 | <0.001* |
| 5 versus 2 | 1.000 | 1.000 | 0.012* |
| 4 versus 3 | 1.000 | 0.113 | 0.040* |
| 5 versus 3 | 1.000 | 1.000 | 1.000 |
| 5 versus 4 | 1.000 | 0.055 | 0.172 |

* ($p < 0.05$)**Supplementary Table S7:** Comparison of *Mycobacterium* spp. gene copies across all plants

| WWTP | <i>P</i> value | | |
|------------|----------------|------------------|--------------|
| | Influent | Activated sludge | SST effluent |
| 2 versus 1 | 1.000 | 1.000 | 1.000 |
| 3 versus 1 | 1.000 | 1.000 | 1.000 |
| 4 versus 1 | 1.000 | 0.581 | 0.255 |
| 5 versus 1 | 1.000 | 1.000 | 1.000 |
| 3 versus 2 | 1.000 | 1.000 | 1.000 |
| 4 versus 2 | 1.000 | 0.310 | 0.080 |
| 5 versus 2 | 1.000 | 1.000 | 1.000 |
| 4 versus 3 | 1.000 | 0.155 | 0.161 |
| 5 versus 3 | 1.000 | 1.000 | 1.000 |
| 5 versus 4 | 1.000 | 0.455 | 0.008* |

* ($p < 0.05$)**Supplementary Table S8:** Comparison of *A. butzleri* gene copies across all plants

| WWTP | <i>P</i> value | | |
|------------|----------------|------------------|--------------|
| | Influent | Activated sludge | SST effluent |
| 2 versus 1 | 1.000 | 1.000 | 0.023* |
| 3 versus 1 | 1.000 | 1.000 | 1.000 |
| 4 versus 1 | 1.000 | 1.000 | 0.022* |
| 5 versus 1 | 1.000 | 0.959 | <0.001* |
| 3 versus 2 | 1.000 | 0.131 | <0.001* |
| 4 versus 2 | 1.000 | 0.678 | <0.001* |
| 5 versus 2 | 1.000 | 1.000 | 1.000 |
| 4 versus 3 | 1.000 | 1.000 | 1.000 |
| 5 versus 3 | 1.000 | 0.116 | <0.001* |
| 5 versus 4 | 1.000 | 0.569 | <0.001* |

* ($p < 0.05$)

Supplementary Table S9: Comparison of *A. hydrophila* gene copies across all plants

| WWTP | <i>P</i> value | | |
|------------|----------------|------------------|--------------|
| | Influent | Activated sludge | SST effluent |
| 2 versus 1 | 1.000 | 1.000 | 0.682 |
| 3 versus 1 | 1.000 | 1.000 | 1.000 |
| 4 versus 1 | 1.000 | 1.000 | 1.000 |
| 5 versus 1 | 1.000 | 0.192 | 0.029* |
| 3 versus 2 | 1.000 | 0.624 | 0.036* |
| 4 versus 2 | 1.000 | 1.000 | 0.274 |
| 5 versus 2 | 1.000 | 1.000 | 1.000 |
| 4 versus 3 | 1.000 | 1.000 | 1.000 |
| 5 versus 3 | 1.000 | 0.022* | 0.001* |
| 5 versus 4 | 1.000 | 0.621 | 0.009* |

* ($p < 0.05$)