Supplementary data

Cycling Conditions for Pathogen directed qPCR

**TaqMan**
(Primer concentration 100 µM)

**M. catarrhalis:**
Hold: 95°C for 10 minutes.

40 Cycles: 95°C for 15 seconds (Not acquiring), 60°C for 45 seconds (Acquiring to Cycling A yellow), 72°C for 20 seconds (Not acquiring).

**P. aeruginosa:**
Hold: 95°C for 10 minutes.

40 Cycles: 95°C for 30 seconds (Not acquiring), 60°C for 60 seconds (Acquiring to Cycling A green).

**S. pneumoniae:**
Hold: 95°C for 10 minutes.

40 Cycles: 95°C for 15 seconds (Not acquiring), 60°C for 45 seconds (Acquiring to Cycling A red), 72°C for 20 seconds (Not acquiring).

**SYBR Green**
(Primer concentration 100 µM)

**H. influenzae and S. aureus:**
Hold: 95°C for 10 minutes.

40 Cycles: 95°C for 20 seconds (Not acquiring), 60°C for 30 seconds (Not acquiring), 72°C for 20 seconds (Acquiring to Cycling A green), 76°C for 20 seconds (Acquiring to Cycling B green).

Melt: 59-99°C with 1°C increase each step, 90 seconds pre-melt, 5 seconds per step.
<table>
<thead>
<tr>
<th>AMR gene</th>
<th>Primers</th>
<th>Annealing Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AcrA-05</strong></td>
<td>Forward: CGTGCGCGGAACGAACA&lt;br&gt;Reverse: ACTTTGC CGCCCATCTTC</td>
<td>60°C</td>
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<tr>
<td><strong>AMPc-04</strong></td>
<td>Forward: TCCGGTGACC GCACAGA&lt;br&gt;Reverse: CAGCAC GC CGGTGAAGT</td>
<td>60°C</td>
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<tr>
<td><strong>cfx-A</strong></td>
<td>Forward: TCATTCCCTCGTTCAAGTTTCAGA&lt;br&gt;Reverse: TGCAGCACCAAGAGGAGATGT</td>
<td>60°C</td>
</tr>
<tr>
<td><strong>FOX-5</strong></td>
<td>Forward: GGT TTGCCGC TGCA GTTC&lt;br&gt;Reverse: GCGCCAGGTGACCA</td>
<td>60°C</td>
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<tr>
<td><strong>PBP2X</strong></td>
<td>Forward: TTCATAAGTATCTGGACATGGAAGAA&lt;br&gt;Reverse: CCAAGGAAACTTGCTTGAGATTAG</td>
<td>60°C</td>
</tr>
<tr>
<td><strong>TetA-01</strong></td>
<td>Forward: GCTGTGGTTCTGCCC GAAA&lt;br&gt;Reverse: GGTTAAGTCTTGA CGCA AACT</td>
<td>60°C</td>
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</tbody>
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Table 1: AMR targeted qPCR primers for additional genes excluded from further analyses