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.

AMR gene	Primers	Annealing Temperature
AcrA-05	Forward: CGTGCGCGAACGAACA Reverse: ACTTTGCGCGCCATCTTC	60^0C
AMPc-04	Forward: TCCGGTGACCGCACAGA Reverse: CAGCACGCCGGTGAAAGT	60^0C
cfx-A	Forward: TCATTCCTCGTTCAAGTTTTCAGA Reverse: TGCAGCACCAAGAGGAGATGT	60^0C
FOX-5	Forward: GGTTTGCCGCTGCAGTTC Reverse: GCGGCCAGGTGACCAA	60^0C
PBP2X	Forward: TTTCATAAGTATCTGGACATGGAAGAA Reverse: CCAAAGGAAACTTGCTTGAGATTAG	60^0C
TetA-01	Forward: GCTGTTTGTTCTGCCGGAAA Reverse: GGTTAAGTTCCTTGAACGCAAACT	60^0C

Table 1: AMR targeted qPCR primers for additional genes excluded from further analyses