

1 High Throughput qPCR validation and controls

2 The PCR mixture for this assay consisted of 50 nl of 2×LightCycler 480 SYBR® Green I Master Mix (Roche
3 Inc., USA), 10 nl of each forward and reverse primers with the final concentration of 1 µM, 20 nl of
4 DNase and RNase free distilled water and finally 10 nl of DNA template making the final reaction
5 volume 100 nl. A non-template control was included per chip. Cycling conditions were described
6 previously.[1] Samples with multiple melting peaks as well as amplification efficiency beyond the
7 range 1·8-2·2 were discarded. The absolute copy number of 16S rRNA gene was quantified separately
8 by Roche 480 using a SYBR® Green approach. *Moraxella catarrhalis* genomic DNA was used as standard
9 and the same primers and cycling conditions, used in high-throughput qPCR were used for this assay.

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11 A total of fourteen chips with a 16 (samples) x 296 (assays) format were used. In each chip 15 DNA
12 extracts plus one non-template control were amplified. Each sample was run as a single replicate. As
13 validation of the methodology four ARG primer pairs were continually positive in the non-
14 template control and were therefore discarded from the analysis. Additionally, two multidrug
15 resistant *Klebsiella pneumoniae* with known ARG profiles were used as positive controls and for these
16 the expected ARG profile was obtained. Three concentrations of human genomic DNA (Bioline) of
17 $2·8 \times 10^2$, $2·8 \times 10^3$ and $2·8 \times 10^4$ genomes/µl respectively were used to determine whether a high
18 background contamination of human DNA could cause false positives in ARG amplification; no
19 interference was observed by the presence of human DNA in the amplification of ARGs detected in
20 this study. Finally, a single background contamination control of the DNA extraction procedure was
21 included for which no ARGs were detected. A threshold cycle (Ct) less than 31 was used as the
22 detection limit based on the previous studies.[1-8]

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24 Though each sample was run as a single replicate internal controls support the strength of data. β -
25 globin and actin, human housekeeping genes, showed the significant correlation which would be
26 expected (figS1 A). Moreover, there were strong correlations observed among a number of other

27 genes which would be predicted to be linked figS1 B-H). Such links could be due to them being in the
28 same operon (*mefA* and *matA/mel*), upon the same mobile genomic element (*ermB* and *tetM*), or by
29 them being mosaic genes (*tetO* and *tetW*).

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34 **Table S1: Correlation between prevalence of ARGs per subject and clinical parameters in a) all**
 35 **subjects b) subjects that received the antibiotics amoxicillin or co-amoxiclav.**

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A		Stable	Exacerbation	Recovery	$\Delta\Delta$ Stable-Exacerbation	$\Delta\Delta$ Exacerbation-Recovery
Post-bronchodilator FEV ₁ , L *	r	0.07	0.12	-0.11	-0.02	-0.23
	p value	0.59	0.41	0.41	0.91	0.10
mCRQ**	r	-0.07	-0.16	0.13	-0.26	-0.18
	p value	0.61	0.26	0.35	0.05	0.20
Total VAS***	r	0.11	-0.17	-0.17	0.06	0.11
	p value	0.44	0.21	0.21	0.70	0.41

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B		Stable	Exacerbation	Recovery	$\Delta\Delta$ Stable-Exacerbation	$\Delta\Delta$ Exacerbation-Recovery
Post-bronchodilator FEV ₁ , L *	r	-0.05	-0.13	-0.27	-0.22	-0.38
	p value	0.79	0.46	0.12	0.21	0.03
mCRQ	r	-0.17	-0.21	0.00	-0.25	-0.34
	p value	0.33	0.22	0.98	0.15	0.04
Total VAS	r	0.20	-0.12	-0.14	0.23	0.08
	p value	0.28	0.51	0.42	0.23	0.65

38 * FEV₁ = forced expiratory volume in 1 second ; ** CRQ = Chronic Respiratory Disease Questionnaire score;

39 ***VAS = visual analog score

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52 **Table S2. List of Individual genes for each ARG family in the same order as present in figure 2 & S2**
 53 **heatmaps. MLSB, Macrolide-Lincosamide-Streptogramin B resistance**

β-lactam	MLSB	Multidrug	Tetracycline
<i>ampC-01</i>	<i>erm(36)</i>	<i>acrA-01</i>	<i>tet(32)</i>
<i>ampC-04</i>	<i>ermA/ermTR</i>	<i>acrA-04</i>	<i>tet(37)</i>
<i>ampC/blaDHA</i>	<i>ermB</i>	<i>acrA-05</i>	<i>tetA-01</i>
<i>bla-L1</i>	<i>ermC</i>	<i>acrF</i>	<i>tetB-01</i>
<i>blaCMY2-01</i>	<i>ermF</i>	<i>ceoA</i>	<i>tetB-02</i>
<i>blaCMY2-02</i>	<i>ermX</i>	<i>emrD</i>	<i>tetC-01</i>
<i>blaCTX-M-02</i>	<i>matA/mel</i>	<i>mepA</i>	<i>tetC-02</i>
<i>blaCTX-M-04</i>	<i>mefA</i>	<i>mexE</i>	<i>tetG-02</i>
<i>blaOXY</i>	<i>mphA-01</i>	<i>mexF</i>	<i>tetK</i>
<i>blaSFO</i>	<i>mphA-02</i>	<i>mtrD-02</i>	<i>tetM-01</i>
<i>blaTEM</i>	<i>msrA-01</i>	<i>mtrD-03</i>	<i>tetM-02</i>
<i>blaZ</i>	<i>pikR2</i>	<i>oprD</i>	<i>tetO-01</i>
<i>cfiA</i>		<i>oprJ</i>	<i>tetPB-02</i>
<i>cfxA</i>		<i>pmrA</i>	<i>tetQ</i>
<i>cphA-01</i>		<i>qacEdelta1-02</i>	<i>tetR-02</i>
<i>cphA-02</i>		<i>qacH-01</i>	<i>tetR-03</i>
<i>fox5</i>		<i>tolC-02</i>	<i>tetW-01</i>
<i>pbp2x</i>		<i>tolC-03</i>	
		<i>yceL/mdtH-01</i>	
		<i>yidY/mdtL-01</i>	

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