High Throughput qPCR validation and controls

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

A total of fourteen chips with a 16 (samples) x 296 (assays) format were used. In each chip 15 DNA extracts plus one non-template control were amplified. Each sample was run as a single replicate. As validation of the methodology four ARG primer pairs were continually positive in the non-template control and were therefore discarded from the analysis. Additionally, two multidrug resistant *Klebsiella pneumoniae* with known ARG profiles were used as positive controls and for these the expected ARG profile was obtained. Three concentrations of human genomic DNA (Bioline) of $2{\cdot}8x10^2$, $2{\cdot}8x10^3$ and $2{\cdot}8x10^4$ genomes/µl respectively were used to determine whether a high background contamination of human DNA could cause false positives in ARG amplification; no interference was observed by the presence of human DNA in the amplification of ARGs detected in this study. Finally, a single background contamination control of the DNA extraction procedure was included for which no ARGs were detected. A threshold cycle (Ct) less than 31 was used as the detection limit based on the previous studies.[1-8]

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

Ramsheh MY, *et al. Thorax* 2019;0:1–9. doi: 10.1136/thoraxjnl-2019-213485

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

Ramsheh MY, *et al. Thorax* 2019;0:1–9. doi: 10.1136/thoraxjnl-2019-213485

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

A		**Stable**	**Exacerbation**	**Recovery**	**ΔΔ Stable-Exacerbation**	**ΔΔ 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

B		**Stable**	**Exacerbation**	**Recovery**	**ΔΔ Stable-Exacerbation**	**ΔΔ 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

* FEV_1 = forced expiratory volume in 1 second ; ** CRQ = Chronic Respiratory Disease Questionnaire score;
***VAS = visual analog score

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

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

References

1. Xu, L., et al., *High-throughput profiling of antibiotic resistance genes in drinking water treatment plants and distribution systems.* Environ Pollut, 2016. **213**: p. 119-26.
2. Wang, F.H., et al., *High throughput profiling of antibiotic resistance genes in urban park soils with reclaimed water irrigation.* Environ Sci Technol, 2014. **48**(16): p. 9079-85.
3. Ouyang, W.Y., et al., *Increased levels of antibiotic resistance in urban stream of Jiulongjiang River, China.* Appl Microbiol Biotechnol, 2015. **99**(13): p. 5697-707.
4. Chen, Q.L., et al., *An underappreciated hotspot of antibiotic resistance: The groundwater near the municipal solid waste landfill.* Sci Total Environ, 2017. **609**: p. 966-973.
5. Chen, Q.-L., et al., *Do manure-borne or indigenous soil microorganisms influence the spread of antibiotic resistance genes in manured soil?* Soil Biology and Biochemistry, 2017. **114**: p. 229-237.
6. Zhu, Y.G., et al., *Continental-scale pollution of estuaries with antibiotic resistance genes.* Nat Microbiol, 2017. **2**: p. 16270.
7. Zhao, Y., et al., *Feed additives shift gut microbiota and enrich antibiotic resistance in swine gut.* Sci Total Environ, 2018. **621**: p. 1224-1232.
8. Chen, Q.-L., et al., *Effect of biochar amendment on the alleviation of antibiotic resistance in soil and phyllosphere of Brassica chinensis L.* Soil Biology and Biochemistry, 2018. **119**: p. 74-82.