**ONLINE SUPPLEMENT**

**TITLE**

Carriage and transmission of macrolide resistance genes in patients with chronic respiratory conditions and their close contacts

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**e-Appendix 1- *Inclusion and exclusion criteria for subject recruitment***

**Macrolide recipient (MR)**

* Inclusion criteria
  1. Age: 18 and above
  2. Must be able to provide written consent to participate
  3. Has at least one chronic lung disease including cystic fibrosis, asthma, or bronchiectasis
  4. Has been on azithromycin or erythromycin maintenance therapy for at least the preceding 6 months
* Exclusion criteria
  1. Unable to provide oropharyngeal swab sample for any reason
  2. Unable to accurately demonstrate / recall antibiotic exposure

**Macrolide non-recipient (MNR)**

* Inclusion criteria
  1. Age: 18 and above
  2. Must be able to provide written consent to participate
  3. Has at least one chronic lung disease including cystic fibrosis, asthma, or bronchiectasis
  4. Is not currently on azithromycin/erythromycin maintenance therapy and has not received any macrolide antibiotics in the prior 6 months
* Exclusion criteria
  1. Unable to provide oropharyngeal swab sample for any reason
  2. Unable to accurately demonstrate / recall antibiotic exposure

**Macrolide recipient close contacts (MRCC) and macrolide non-recipients close contacts (MNRCC)**

* Inclusion criteria
  1. Must be able to provide written consent to participate
  2. Is either a close household contact (Spouse, defacto or family members) who has lived with the patient for the immediate proceeding period at least 6 months or was a close family member (parent or sibling or partner) or friend who has had close contact with the patient over the immediate proceeding period of 2 years, as defined by at least 2 times a week.
  3. Have not received any antibiotics (any class) in the prior 4 weeks
  4. Has not received any macrolide in the prior 6 months and has not received any azithromycin in the prior 12 months
  5. No chronic respiratory disease, excepting well-controlled asthma
  6. Has not been treated as a patient in hospital in the past 4 weeks
* Exclusion criteria
  1. Unable to provide oropharyngeal swab sample for any reason
  2. Unable to accurately demonstrate / recall antibiotic exposure

**e-Appendix 2 - *Quantitation of total bacterial load, resistance gene carriage***

Quantitative PCR (qPCR) assays were employed to quantify the total bacterial load (16S qPCR), detect resistance genes, and quantify abundance of resistance genes in each sample. The SYBR Green method was employed for measuring CT values of six macrolide resistance genes [*erm*(A), *erm*(B), *erm*(C), *erm*(F), *msr*(A),and *msr*(*E*)] and three tetracycline resistance genes [*tetM*, *tetO*, and *tetW*]. A Taqman assay was used for assessing CT values of the macrolide resistance gene *mef.* Each reaction using the SYBR Green method was prepared with 17.5 µL 2×PowerUp SYBGreen Master Mix (Applied Biosystems, Foster City, CA, United States), 15.1 µL of Nuclease-Free water, 0.7 µL of 10 µM forward primer, 0.7 µl of 10 µM reverse primer, mixed with 1 µl of DNA template. Each reaction using the Taqman method was prepared with 17.5 µL 2×Kappa Fast probe low rox (KAPA Biosystems, Woburn, MA, United States), 14.05 µL of Nuclease-Free water, 0.875 µL of 10 µM forward primer, 0.875 µl of 10 µM reverse primer, 0.7 µL of µM probe, mixed with 1 µl of DNA template. The annealing temperature of 16S rRNA gene, *erm*(A), *erm*(B), *erm*(C), *erm*(F), *msr*(A), *msr*(*E*), *tetM* and *tetW* gene was 60 °C. The annealing temperature of *mef* and *tetO* gene was 62 °C.

**e-Table 1.** Quantitative PCR primers and probes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Detection limit (Ct value)** | **Primer** | **Amplicon size (bp)** | **Reference** |
| **16S** | 27.50 | F: 5'-TCCTACGGGAGGCAGCAGT-3'  R: 5'-GGACTACCAGGGTATCTAATCCTGTT-3' | 467 | (E1) |
| ***erm*(A)** | 34.26 | F: 5'-TCAGTTACTGCTATAGAAATTGATGGAG-3'  R: 5'-ATACAGAGTCTACACTTGGCTTAGG-3' | 358 | (E2) |
| ***erm*(B)** | 34.64 | F: 5'-GAAAGCCRTGCGTCTGACATC-3'  R: 5'-CGAGACTTGAGTGTGCAAGAGC-3' | 105 | (E3) |
| ***erm*(C)** | 33.40 | F: 5'-CTTGTTGATCACGATAATTTCC-3'  R: 5'-ATCTTTTAGCAAACCCGTATTC-3' | 190 | (E4) |
| ***erm*(F)** | 32.59 | F: 5'-CGGGTCAGCACTTTACTATTG-3'  R: 5'-GGACCTACCTCATAGACAAG-3' | 466 | (E5, E6) |
| ***msr*(A)** | 34.04 | F: 5'-TCCAATCATTGCACAAAATCTAAC-3'  R: 5'-TCAATTCCCTCTATTTGGTGGT-3' | 165 | (E4) |
| ***msr*(E)** | 36.74 | F: 5'-TCGATACGAAGAGGCGATGC-3'  R: 5'-CTTCTGTTTGGTGCCGGTTG-3' | 163 | (E7) |
| ***tetM*** | 33.82 | F: 5'-CAGAATTAGGAAGCGTGGACAA-3'  R: 5'-CCTCTCTGACGTTCTAAAAGCGTAT-3' | 67 | (E8) |
| ***tetO*** | 31.96 | F: 5'-AACTTAGGCATTCTGGCTCAC-3'  R: 5'-TCCCACTGTTCCATATCGTCA-3' | 515 | (E3) |
| ***tetW*** | 31.31 | F: 5'-GAGAGCCTGCTATATGCCAGC-3'  R: 5'-GGGCGTATCCACAATGTTAAC-3' | 168 | (E9) |
| ***mef*** | 37.14 | F: 5'-TATGGAGCTACCTGTCTGGA-3'  R: 5'-GGTACTAAAAGTGGCGTAACC-3'  Probe: HEX-CCGTAGCATTGGAACAGCTTTTC-BHQ1 | 85 | (E10) |

F, forward primer; R, reverse primer; cycle number,40;

**e-Table 2.** Resistance gene detection frequency and relative abundance in close contacts of macrolide recipient and macrolide non-recipients.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Detection limit**  **(Ct value)** | **% of close contact**  **carried the gene** | | ***P* values** | **Normalised gene levels** | | ***P* values** |
| **MRCC** | **MNRCC** | **MRCC** | **MNRCC** |
| ***erm*(A)** | 34.26 | 0%  (0/53) | 0%  (0/40) | 0.99 | 0  (0.0-0.0) | 0  (0.0-0.0) | 0.50 |
| ***erm*(B)** | 34.64 | 85%  (45/53) | 85%  (34/40) | 0.99 | 3.4  (0.0-10.8) | 4.2  (0.0-10.1) | 0.48 |
| ***erm*(C)** | 33.40 | 7.5%  (4/53) | 5.0%  (2/40) | 0.70 | 0  (0.0-11.1) | 0  (0.0-9.9) | 0.42 |
| ***erm*(F)** | 32.59 | 45%  (24/53) | 65%  (26/40) | 0.09 | 0  (0.0-12.3) | 3.3  (0.0-9.9) | 0.065 |
| ***mef*** | 37.14 | 62%  (33/53) | 63%  (25/40) | 0.58 | 1.3  (0.0-5.6) | 1.2  (0.0-4.7) | 0.38 |
| ***msr*(A)** | 34.04 | 36%  (19/53) | 30%  (12/40) | 0.66 | 0  (0.0-12.7) | 0  (0.0-9.1) | 0.26 |
| ***msr*(E)** | 36.74 | 36%  (19/53) | 48%  (19/40) | 0.29 | 0  (0.0-13.5) | 0  (0.0-19.7) | 0.25 |
| ***tetM*** | 33.82 | 96%  (51/53) | 95%  (38/40) | 0.99 | 4.9  (2.3) | 4.4  (1.9) | 0.12 |
| ***tetO*** | 31.96 | 62%  (33/53) | 65%  (26/40) | 0.83 | 4.0  (0.0-10.0) | 4.9  (0.0-10.1) | 0.41 |
| ***tetW*** | 31.31 | 77%  (41/53) | 80%  (32/40) | 0.80 | 4.7  (0.0-11.3) | 5.0  (0.0-8.9) | 0.47 |

Abbreviations: MRCC: close contacts of patients who were receiving long-term macrolide therapy; MNRCC close contacts of patients who were not receiving any macrolide therapy. *P* value for detection frequency was determined by Fisher's exact test; *P* values for gene levels comparisons were determined by Mann-Whitney U test (non-parametric data, one-tailed test) and unpaired Student’s t test with Welch's correction (parametric data, one-tailed test). Close contact defined as either a close household contact (Spouse, defacto or family members) who has lived with the patient for the immediate proceeding period at least 6 months or is a close family member (parent or sibling or partner) or friend who has had close contact with the patient over the immediate proceeding period of 2 years, as defined by at least 2 times a week.

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**e-Figure 1.** **Study design**

Subjects were recruited by respiratory physicians. Macrolide recipients: patients who receive macrolide maintenance therapy; Macrolide non-recipients: patients who did not received macrolide maintenance therapy; Close contact defined as either a close household contact (Spouse, defacto or family members) who has lived with the patient for the immediate proceeding period at least 6 months or is a close family member (parent or sibling or partner) or friend who has had close contact with the patient over the immediate proceeding period of 2 years, as defined by at least 2 times a week. All patients were categorized based on their primary diagnosis

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**e-Figure 2.** **Resistance gene presence/absence map.**

Gene detection (red) determined by a qPCR amplification signal greater than the lowest positive serial dilution; P: patients; CC: paired close contact of patient; MR group: Macrolide recipient group; MNR group: Macrolide non-recipient group