**ONLINE SUPPLEMENT**

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

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***Inclusion and exclusion criteria for subject recruitment***

**AZM patient**

* **Inclusion criteria**

1. Age: 18-40 years
2. Must be able to provide written consent to participate
3. Has a chronic lung disease including cystic fibrosis, asthma, or bronchiectasis
4. Is currently on azithromycin maintenance therapy at a dose greater than 500 mg/week
5. Has been on azithromycin maintenance therapy for at least 6 months for at least 50% of the time
6. At least 50% medical compliance verified by evidence of filled scripts on pharmacy records

* **Exclusion criteria**

1. Unable to provide oropharyngeal swab sample for any reason
2. Unable to accurately demonstrate / recall antibiotic exposure
3. Vulnerable subjects

* **Medication permitted**

1. Inhaled/nebulised antibiotics in chronic, daily stable dose
2. Inhaled steroids in chronic, daily stable dose
3. Inhaled/nebulized mucolytic therapies (hypertonic saline, mannitol, dornase alpha, N-acetylcysteine)

**Non-AZM patient**

* **Inclusion criteria**

1. Age: 18-40 years
2. Must be able to provide written consent to participate
3. Has a chronic lung disease including cystic fibrosis, asthma, or bronchiectasis
4. Is not currently on azithromycin maintenance therapy and has not received any azithromycin therapy in the last 2 years
5. Has not received a macrolide other than azithromycin in the last 1 year

* **Exclusion criteria**

1. Unable to provide oropharyngeal swab sample for any reason
2. Unable to accurately demonstrate / recall antibiotic exposure
3. Vulnerable subjects

* **Medication permitted**

1. Inhaled/nebulised antibiotics in chronic, daily stable dose
2. Inhaled steroids in chronic, daily stable dose
3. Inhaled/nebulized mucolytic therapies (hypertonic saline, mannitol, dornase alpha, N-acetylcysteine)

**AZM close contact and non-AZM close contact**

* **Inclusion criteria**

1. Age: 18-85 years
2. Must be able to provide written consent to participate
3. Is either a close household contact 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
4. Has never received azithromycin
5. 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
3. Vulnerable subjects

***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 [*tet*(M), *tet*(O), and *tet*(W)]. 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*), *tet*(M) and *tet*(W) gene was 60 °C. The annealing temperature of *mef* and *tet*(O) gene was 62 °C.

**Table E1.** Quantitative PCR primers and probes

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Primer** | **Amplicon size (bp)** | **Reference** |
| **16S** | F: 5'-TCCTACGGGAGGCAGCAGT-3'  R: 5'-GGACTACCAGGGTATCTAATCCTGTT-3' | 467 | (E1) |
| ***erm*(A)** | F: 5'-TCAGTTACTGCTATAGAAATTGATGGAG-3'  R: 5'-ATACAGAGTCTACACTTGGCTTAGG-3' | 358 | (E2) |
| ***erm*(B)** | F: 5'-GAAAGCCRTGCGTCTGACATC-3'  R: 5'-CGAGACTTGAGTGTGCAAGAGC-3' | 105 | (E3) |
| ***erm*(C)** | F: 5'-CTTGTTGATCACGATAATTTCC-3'  R: 5'-ATCTTTTAGCAAACCCGTATTC-3' | 190 | (E4) |
| ***erm*(F)** | F: 5'-CGGGTCAGCACTTTACTATTG-3'  R: 5'-GGACCTACCTCATAGACAAG-3' | 466 | (E5, E6) |
| ***msr*(A)** | F: 5'-TCCAATCATTGCACAAAATCTAAC-3'  R: 5'-TCAATTCCCTCTATTTGGTGGT-3' | 165 | (E4) |
| ***msr*(E)** | F: 5'-TCGATACGAAGAGGCGATGC-3'  R: 5'-CTTCTGTTTGGTGCCGGTTG-3' | 163 | (E7) |
| ***tet*(M)** | F: 5'-CAGAATTAGGAAGCGTGGACAA-3'  R: 5'-CCTCTCTGACGTTCTAAAAGCGTAT-3' | 67 | (E8) |
| ***tet*(W)** | F: 5'-GAGAGCCTGCTATATGCCAGC-3'  R: 5'-GGGCGTATCCACAATGTTAAC-3' | 168 | (E9) |
| ***tet*(O)** | F: 5'-AACTTAGGCATTCTGGCTCAC-3'  R: 5'-TCCCACTGTTCCATATCGTCA-3' | 515 | (E3) |
| ***mef*** | F: 5'-TATGGAGCTACCTGTCTGGA-3'  R: 5'-GGTACTAAAAGTGGCGTAACC-3'  Probe: HEX-CCGTAGCATTGGAACAGCTTTTC-BHQ1 | 85 | (E10) |

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

**Table E2.** Gene detection frequency in AZM/non-AZM close contacts

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Resistance gene** | **Detection limit**  **(Ct value)** | **AZM**  **close contacts** | **Non-AZM**  **close contacts** | ***P* value** |
| ***erm*(A)** | 32.97 | 0%  (0/35) | 0%  (0/17) | >0.99 |
| ***erm*(B)** | 34.64 | 80%  (28/35) | 94%  (16/17) | 0.25 |
| ***erm*(C)** | 33.40 | 2.9%  (1/35) | 0%  (0/17) | >0.99 |
| ***erm*(F)** | 31.92 | 31%  (11/35) | 71%  (12/17) | **0.016** |
| ***mef*** | 39.83 | 74%  (26/35) | 82%  (14/17) | 0.73 |
| ***msr*(A)** | 32.56 | 8.6%  (3/35) | 5.9%  (1/17) | >0.99 |
| ***msr*(E)** | 36.77 | 40%  (14/35) | 53%  (9/17) | 0.55 |
| ***tet*(M)** | 34.90 | 97%  (34/35) | 100%  (17/17) | >0.99 |
| ***tet*(O)** | 31.01 | 60%  (21/35) | 59%  (10/17) | >0.99 |
| ***tet*(W)** | 33.41 | 94%  (33/35) | 88%  (15/17) | 0.59 |

*P* value determined by Fisher's exact test; AZM: azithromycin; Close contact: a close household contact who has lived with a patient with a respiratory disease for at least six months, or a close family member or friend who has had contact with the patients ≥2 times a week over the last two years.

**REFERENCE**

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**Figure E1. Study design**

Subjects were recruited by respiratory physicians. AZM patients: patients who receive azithromycin maintenance therapy; Non-AZM patients: patients who did not received azithromycin maintenance therapy; Close contact: a close household contact who has lived with a patient with a respiratory disease for at least six months, or a close family member or friend who has had contact with the patients ≥2 times a week over the last two years. All patients were categorized based on their primary diagnosis

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**Figure E2. The distribution of 16S gene copies in each cohort.**

Total bacterial load was determined using a qPCR assay targeting a conserved region of the 16S rRNA gene. AZM: azithromycin; Close contact: a close household contact who has lived with a patient with a respiratory disease for at least six months, or a close family member or friend who has had contact with the patients ≥2 times a week over the last two years. Showing mean and standard deviation; significance calculated by Kruskal-Wallis with Dunn’s post-hoc test.

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**Figure E3. Resistance gene presence/absence map.**

Gene detection (red) determined by a qPCR amplification signal greater than the lowest positive serial dilution; AZM: azithromycin; P: patients; CC: paired close contact of patient