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

Yiming Wang1,2, Steven L Taylor1,2#,Jocelyn M Choo1,2, Lito E. Papanicolas1,2, Rebecca Keating3, Kate Hindmarsh4, Geraint B. Rogers1,2†, Lucy D. Burr3,4†

1. Microbiome Research Laboratory, College of Medicine and Public Health, Flinders University, Adelaide, Australia

2. Microbiome & Host Health, South Australia Health and Medical Research Institute, North Terrace, Adelaide, Australia

3. Department of Respiratory Medicine, Mater Health Services, South Brisbane, QLD,

Australia

4. Mater Research - University of Queensland, Aubigny Place, South Brisbane, QLD, Australia

† Joint senior author

# Corresponding author:  Dr Steven Taylor

5D305 Flinders Medical Centre,

Flinders Drive BEDFORD PARK,

South Australia 5042

+618 8204 5711

[steven.taylor@sahmri.com](mailto:steven.taylor@sahmri.com)

**RUNNING TITLE**

AMR in macrolide recipients and contacts

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**DISCLOSURE STATEMENT**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

**AUTHOR’S CONTRIBUTIONS**

The study was conceived by GBR and LDB. LDB, RK, KH were responsible for subject recruitment and sample collection. YW was responsible for sample processing and data generation. YW, SLT, GBR undertook the data analysis. YW, SLT, GBR, drafted the manuscript, with the support of JMC and LEP. All authors approved the final version of the manuscript.

**KEY WORDS:** macrolides, antibiotic resistance, asthma, cystic fibrosis, azithromycin

**WORD COUNT:** 2401

**ABSTRACT**

**Rationale:** Long-term azithromycin therapy has been shown to provide substantial clinical benefit in patients with a range of chronic respiratory conditions. However, the impact of azithromycin exposure on the carriage and abundance of antibiotic resistance genes within the oropharynx is poorly defined. The potential for onward transmission of resistance from azithromycin recipients to close recipient contacts is also not well understood.

**Objective:** To determine the impact of long-term azithromycin therapy on carriage of resistance determinants within the oropharyngeal microbiota in patients with chronic respiratory conditions, and assess evidence of onward transmission of resistance genes to close patient contacts.

**Methods:** Oropharyngeal swabs were collected from 52 individuals with chronic respiratory conditions (44 cystic fibrosis, 8 asthma), of whom 35 were receiving long-term azithromycin. For each subject, an additional oropharyngeal swab was collected from a close co-habiting contact. The detection of ten macrolide-associated resistance genes was determined by quantitative PCR.

**Results:** The detection of macrolide-associated resistance genes was not significantly more common in recipients of azithromycin compared to non-recipients. However, the normalised gene abundance of *mef* was higher in the azithromycin group than in the non-azithromycin group (p<0.0001). In patient co-habitants, only *erm*(F) detection was significantly different between groups (higher for non-azithromycin close contacts, p=0.016). Paired assessment of normalised gene abundance showed *erm*(F), *mef*, and *msr*(E) to be significantly more abundant in azithromycin recipients compared to their close contacts, with no such relationships within the non-azithromycin group. Detection of *erm*(F) in azithromycin recipients, but not non-recipients, was significantly associated with detection in close contacts (p=0.013).

**Conclusions:** We report increased macrolide gene relative abundance in patients receiving azithromycin, supporting previous studies. However, azithromycin use was not associated with increased macrolide resistance gene detection and there was limited evidence that patient close contacts are at increased risk of resistance acquisition.

**Clinical Trial Registration:** HREC/14/MHS/68

**Word Count:** 301

**INTRODUCTION**

Azithromycin is a 15-membered-ring macrolide antibiotic with a diverse range of clinical uses. In addition to the treatment of respiratory (1, 2) and soft-tissue infections (3), azithromycin is employed as part of prophylaxis measures in caesarean deliveries (4-6), and to reduce community rates of *Chlamydia trachomatis* infection in resource-limited countries (7). Azithromycin is also being used increasingly in the management of chronic respiratory conditions, including cystic fibrosis (8-12), severe uncontrolled asthma (13-15), chronic obstructive pulmonary disease (16-18), and bronchiectasis (19-21).

While long-term treatment with azithromycin in chronic respiratory disease appears to be both safe and effective (10, 11, 13, 20), the potential for increased carriage of macrolide resistance is a potential concern (22-25). Little is known currently about the potential for increased antibiotic resistance gene carriage in those receiving azithromycin for chronic respiratory conditions to contribute to population-level resistance.

We hypothesised that the carriage of macrolide resistance genes, and associated resistance genes under co-selection, would be more frequent within the oropharyngeal microbiota of recipients of long-term azithromycin for chronic respiratory conditions, compared to azithromycin-naïve patients. We further hypothesised that any differences in resistance carriage between recipient and non-recipient patients would be reflected in resistance carriage rates in patient close contacts, suggesting a potential route for person to person transmission of resistance genes.

We report a quantitative PCR (qPCR)-based assessment of oropharyngeal detection and abundance of 10 macrolide-associated antibiotic resistance genes in 52 patients with chronic respiratory conditions, of whom 35 were receiving long-term azithromycin, and their close contacts.

**METHODS**

**Study design and setting**

A single-blind study design was used to characterise carriage of macrolide-associated antibiotic resistance gene and relative abundance of these genes in the oropharyngeal microbiota of recipients and non-recipients with chronic respiratory conditions (Online Supplement Figure E1). Whether any increase in resistance gene carriage in azithromycin recipients was associated with increased carriage in close contacts, consistent with transmission, was also assessed. The study was approved by the Mater Health Services Human Resource Ethics Committee (HREC/14/MHS/68) in Queensland, Australia. All study participants provided written informed consent. Details of subject inclusion and exclusion criteria are provided in the Online Supplementary methods.

Subjects attending the Mater Adult Cystic Fibrosis service and the Mater Young Adult Respiratory Service were recruited. Close contacts were identified at the time of subject recruitment and were asked to attend the Mater Respiratory Research Unit for sample collection within one week of the index subject (samples were usually collected on the same day).

Subjects were assigned to one of four groups: 1) azithromycin-recipients (AZM patients), defined as those receiving azithromycin maintenance therapy for a chronic respiratory condition at a dose greater than 500 mg/week, and who had received azithromycin for at least 50% of the time over the preceding six months; 2) non-azithromycin recipients (non-AZM patients), defined as those with a chronic respiratory condition who were not receiving azithromycin maintenance therapy, and who had not received azithromycin in the prior two years; 3) AZM-recipient close contacts (AZM close contacts), defined as either a close household contact who has lived with an AZM recipient for at least six months, or a close family member or friend who has had contact with the patients at least two times a week over the last two years; or 4) non-AZM recipient close contacts (non-AZM close contacts), defined as a close contact of a non-AZM recipient. Close contacts had never received azithromycin therapy. AZM close contacts and non-AZM close contacts who had received hospital treatment in the prior four weeks were excluded.

**Sample collection**

Oropharyngeal microbiota sample collection was performed using Copan Amies Transport swabs without charcoal (Interpath Services, Heidelberg West, VIC, AU). Swabs were passed over the tonsils and posterior pharyngeal wall, while avoiding contact with jaws, teeth and gingiva, as described previously by Malhotra-Kumar *et al.*(24). Swabs were stored at -80 °C prior to processing for analysis.

**Sample preparation and DNA extraction**

Swabs were heated at 95 °C for 3 min, vortexed in the collection medium for 30 s, and centrifuged at 13,000 × *g* for 5 min at 30 °C. Pellets were resuspended in 400 µL Tris-EDTA buffer and nucleic acid extraction was performed by using ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA, USA), including beat beating at 6.5 m/s on a FastPrep (MP Biomedicals, Irvine, CA, USA) for 5 min, in accordance with the manufacturer’s instructions. DNA was quantified using a Qubit 2.0 (Invitrogen, Carlsbad, CA, USA).

**Resistance gene detection and relative abundance quantification**

Total bacterial load was determined using a SYBR Green based qPCR assay targeting a conserved region of the 16S rRNA gene (Online Supplement Table E1). Quantification 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) was achieved using SYBR Green based assays. Quantification of the *mef* macrolide resistance genewas achieved using a Taqman-based assay. Oligonucleotides used for each reaction, and amplicon sizes are provided in Online Supplement Table E1. Resistance gene levels were determined by comparison of sample signal with serial dilutions of a positive control (DNA from a bacterial isolate confirmed to harbour the resistance gene). Detection of amplification signal greater than the lowest positive serial dilution was considered positive. Resistance gene abundance was normalised to the 16S Ct value of each sample: ΔCt (ΔCt=Cttarget gene-Ct16S) and transformed so a higher value represents higher gene abundance, as described previously (25). 16S copies were comparable between groups (Figure E2). A detailed description of qPCR methods is provided in Supplementary Methods.

**Statistical analysis**

Assessments of study population characteristics and paired assessment of the resistance gene detection frequency between patients and close contacts were performed in SPSS version 25 (IBM SPSS, Armonk, NY). All other data analysis was performed in GraphPad Prism version 8.2.1 (GraphPad Software Inc., San Diego, CA, USA). Student’s *t* test was used to analyse unpaired parametric data. Mann-Whitney U test was utilized to analyse unpaired non-parametric data. Fisher’s exact test was employed to analyse proportional data. Binary logistic regression was used to analyse paired nominal data.

**RESULTS**

A total of 67 individuals with chronic respiratory conditions were recruited. Of these, 13 were excluded due to failure to recruit a close contact. A further two patients were excluded because they had received clarithromycin treatment in the prior 12 months. Baseline demographic and disease characteristics are shown for both patient and close contact groups in Table 1. While the characteristics of AZM and non-AZM groups were broadly similar, the AZM group had a significantly higher burden of co-morbidities and non-macrolide antibiotic exposure. Close contacts of AZM and non-AZM recipients did not differ significantly (Table 1).

Ten resistance genes were assessed in terms of gene detection (presence or absence), as well as gene relative abundance (gene levels normalised to the bacterial 16S gene). The detection of the ten resistance genes varied substantially, from 100% detection for *tet*(M) (combined patient groups) to 5.8% for *erm*(A). No significant differences were identified in the frequency of oropharyngeal detection of any assessed resistance gene between AZM and non-AZM patients (Table 2). When resistance gene relative abundance was assessed, *mef* was found to be significantly higher in the AZM patients compared to the non-AZM patients (p<0.0001; Figure 1). There were no significant differences in the relative abundance of any of the other resistance genes assessed (Table 3).

The detection of resistance genes in the close contacts of AZM and non-AZM patients was then assessed (Online Supplement Table E2). Again, detection frequency varied substantially between resistance genes, ranging from 98% for *tet*(M) to 0% for *erm*(A). Detection frequency of *erm*(F) was significantly higher for the non-AZM close contacts compared to the AZM close contacts (12/17 and 11/35, respectively; p=0.016). There was no significant difference in the detection frequency of any other resistance genes (Online Supplement Table E2).

Paired comparison of relative abundance of each gene in patients and close contacts was then performed. Levels of *erm*(F), *mef*, and *msr*(E) were found to be significantly higher in AZM treated patients compared to their close contacts. However, no such relationship was observed between non-AZM patients and their close contacts (Figure 2).

Detection of resistance genes in patients and their close contacts is shown in Online Supplement Figure E3. Paired assessment of the resistance gene detection showed the presence of *erm*(F) in AZM close contacts is significantly associated with the presence of *erm*(F) in AZM patients (p=0.013, Table 4). This association was not found for any other genes, nor between patients and close contacts in the non-AZM group (Table 4).

**DISCUSSION**

Our aim was to investigate the impact of long-term azithromycin treatment on carriage of resistance determinants within the oropharyngeal microbiota in patients with chronic respiratory conditions, and assess evidence of onward transmission of resistance genes to close patient contacts. In doing so, we address the potential for substantial increases in the use of macrolide antibiotics in the clinical management of chronic respiratory disease patients to contribute to the growing population-level burden of antibiotic resistance carriage.

The resistance genes assessed here have been demonstrated to be common determinants of macrolide resistance in respiratory bacterial pathogens, or have been shown in prior studies to increase in prevalence within the oropharynx as a result of long-term macrolide exposure (24, 26). Specifically, there were seven genes that confer macrolide resistance via either modification of the ribosomal target site (*erm*(A), *erm*(B), *erm*(C), and *erm*(F)), protection of the target site (*msr*(A) and *msr*(E)), or a macrolide efflux pump (*mef*). All have been shown to be associated with mobile genetic elements (27-34). Further, three tetracycline resistance genes: *tet*(M), *tet*(O), and *tet*(W), were assessed based on their association with azithromycin treatment (24, 25), and carriage on mobile genetic elements alongside macrolide resistance genes (25, 28, 35, 36).

Our subject cohort included adult asthma and CF patients; both groups in which substantial benefit has been demonstrated for long-term azithromycin (8-15). Notably, AZM and non-AZM groups had marked differences in their clinical characteristics and treatment exposures, including a higher frequency of hospital admission for AZM patients and a greater burden of acute non-macrolide antibiotics. However, such disparities are unlikely to influence oropharyngeal carriage of macrolide resistance determinants directly.

Both patients and close contacts were stable at the time of sample collection. The risk of transmission of resistant bacterial populations would therefore be less than during periods of exacerbation. For example, periods of exacerbation can include increased coughing (37, 38), which has been shown to promote dissemination of potentially resistant bacteria through the production of cough aerosols (39, 40). Further, during periods of stability, intact commensal microbial systems, including those in the oropharynx, resist colonisation by external bacterial populations (41). This protection is greatly reduced when microbiota are disrupted, such as during respiratory viral infection (42). The risks of onward transmission of resistant bacterial populations might therefore vary according to the health of both azithromycin recipients and close contacts.

Our investigation of specific resistance genes highlighted the commonness of detection of many of the determinants assessed, both within the oropharyngeal microbiota of patients being treated for chronic respiratory conditions, and within the non-patient population. A previous study by Malhotra-Kumar *et al.* reported that ~80% of oropharyngeal streptococci harbour the *mef* gene, despite subjects being healthy and having no macrolide exposure (24), suggesting carriage of some macrolide resistance determinants may be widespread. Indeed, we detected *mef* in 82% of non-AZM close contacts. We also detected *erm*(B) and *tet*(M) in a high proportion of non-AZM close contacts (94%, and 100%, respectively), again, in keeping with the high relative prevalence of these genes in oropharyngeal streptococci, reported previously (30% and 40%, respectively) (24). As these genes are present on commonly acquired on mobile genetic elements, including conjugative transposons and conjugative plasmids (43, 44), they have been found in multiple organisms, with a high degree of transferability (43, 44).

The absence of substantial differences, either between AZM and non-AZM groups, or between patient groups and their respective close contacts, suggests that macrolide exposure is not associated with increased carriage frequency in the short-term. However, we did see an increase in the relative abundance of *mef* in AZM patients compared to non-AZM patients. This finding supports a previous study where *mef* abundance increased in the oropharynx of patients with bronchiectasis after 48 weeks of erythromycin therapy (26). Such increases in the abundance of resistant bacteria might be expected to increase the likelihood of onward transmission, with the risk of such transmission greatest for those living in close physical proximity. However, our paired assessment of resistance gene relative abundance showed significantly higher gene carriage in AZM patients compared to AZM close contacts, meaning we saw no evidence that such transmission occurs at a substantial level.

Our study did have a number of limitations that should be considered. Analysis was based on resistance gene carriage and did not include phenotypic assessment of resistance. However, each of the genes assessed have been shown previously to confer phenotypic resistance in oropharyngeal pathobionts and common respiratory pathogens. Our analysis did not include determination of shifts in microbiota composition and did not attempt to determine whether increases in the relative abundance of individual resistance genes resulted from increases in the size of pre-existing resistant bacterial populations or through the horizontal transfer of resistance determinants to new populations. Assessment was performed at a resistome level, i.e. the totality of resistance genes carried within the microbial community. The extent to which individual taxa were associated with specific resistance genes was not assessed. Furthermore, resistance conferred by point mutations or amino acid alteration in ribosomal proteins, two common macrolide resistance mechanisms for certain pathogens (45, 46), was not assessed. Finally, it should be noted that the size of the subject groups was not even. The number of patients and close contacts assessed was smaller for the non-AZM than the AZM group, a difference that reflected a lower rate of close contact recruitment in for non-AZM patients.

In summary, we report long-term azithromycin in patients with chronic respiratory conditions to be associated with increased abundance of associated resistance genes within the oropharyngeal microbiota. However, azithromycin exposure was not associated with increased resistance gene carriage frequency. A significant association was identified between carriage of *erm*(F) in patients receiving AZM and carriage by close contacts. However, *erm*(F) carriage was not significantly higher in close contacts of AZM patient compared to non-AZM patients. Taken together, these findings suggest that the association between azithromycin treatment, increased carriage of transmissible macrolide resistance determinants, and their onwards transmission, is not substantial.

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**FIGURE LEGENDS**

**Figure 1. Comparison of *mef* abundance in recipients and non-recipients of azithromycin (AZM).** *mef* abundance normalised to bacterial 16S copies. Bars show mean and standard deviation; significance calculated by Student's t-test with Welch’s correction.

**Figure 2. Comparison of *erm*(B), *erm*(F), *mef*, and *msr*(E) abundance between patients/close contacts pairs**

Gene levels normalised to total bacterial load. Significance of gene abundance calculated by Wilcoxon matched-pairs signed rank test; AZM: azithromycin.

**Table 1.** Study population characteristics

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Characteristic** | **AZM**  **Patients** | **Non-AZM**  **patients** | ***P* value** | **AZM**  **close contacts** | **Non-AZM**  **close contacts** | ***P* value** |
| N | 35 | 17 |  | 35 | 17 |  |
| Respiratory condition n (%)† |  |  |  |  |  |  |
| Cystic fibrosis | 34 (97) | 10 (59) | <0.001 | 0 | 0 | >0.99 |
| Asthma | 1 (2.9) | 7 (41) | <0.001 | 1 (2.9) | 4 (24) | 0.36 |
| Age, years\* |  |  |  |  |  |  |
| Mean (SD) | 32.1 (11.1) | 29.8 (8.8) | 0.45 | 42.9 (12.9) | 37.1 (14.8) | 0.18 |
| Sex, n (%)† |  |  |  |  |  |  |
| Male | 21 (60) | 8 (47) | 0.55 | 10 (29) | 8 (47) | 0.22 |
| Female | 14 (40) | 9 (53) | 0.55 | 25 (71) | 9 (53) | 0.22 |
| Smoking status, n (%)† |  |  |  |  |  |  |
| Non-smoker | 32 (91) | 13 (77) | 0.20 | 21 (60) | 8 (47) | 0.55 |
| Current smoker | 0 | 0 |  | 4 (11) | 3 (18) | 0.66 |
| Ex-smoker | 3 (8.6) | 4 (24) | 0.20 | 10 (29) | 5 (29) | >0.99 |
| Hospital admission in last 4 weeks, n (%)† | 5 (14) | 1 (5.9) | 0.65 | 0 | 0 | >0.99 |
| Macrolide exposure, n (%)† |  |  |  |  |  |  |
| Azithromycin ever | 35 (100) | 3 (18) | <0.001 | 0 | 0 | >0.99 |
| Azithromycin in last 2 years | 35 (100) | 0 | <0.001 | 0 | 0 | >0.99 |
| Azithromycin for more than 1 week | 35 (100) | 3 (18) | <0.001 | 0 | 0 | >0.99 |
| Any other macrolide in the last year | 0 | 0 | >0.99 | 1 (2.9) | 0 | >0.99 |
| Non-macrolide antibiotics in last 4 weeks, n (%)† | 8 (23) | 2 (12) | 0.47 | 1 (2.9) | 0 | >0.99 |
| Nebulised tobramycin | 21 (60) | 0 | <0.001 | 0 | 0 | >0.99 |
| Nebulised colistin | 11 (31) | 0 | 0.010 | 0 | 0 | >0.99 |
| Tazocin (IV) | 4 (11) | 0 | 0.29 | 0 | 0 | >0.99 |
| Azole (Oral) | 3 (8.6) | 1 (5.9) | >0.99 | 0 | 0 | >0.99 |
| Bactrim (Oral) | 6 (17) | 1 (5.9) | >0.99 | 0 | 0 | >0.99 |
| Ciprofloxacin (Oral) | 4 (11) | 1 (5.9) | >0.99 | 0 | 0 | >0.99 |
| Tobramycin (IV) | 3 (8.6) | 0 | 0.54 | 0 | 0 | >0.99 |
| Piperacillin / Tazobactam (IV) | 2 (5.7) | 0 | >0.99 | 0 | 0 | >0.99 |
| Meropenem (IV) | 2 (5.7) | 0 | >0.99 | 0 | 0 | >0.99 |
| Augmentin (Oral) | 0 | 0 | >0.99 | 1 (2.9) | 0 | >0.99 |
| Metronidazole (Oral) | 0 | 1 (5.9) | 0.33 | 0 | 0 | >0.99 |

\* Mean (SD), student's t-test; † n (%), 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. All patients categorized based on primary diagnosis. IV: intravenous

**Table 2.** Resistance gene detection frequency in AZM/non-AZM patients

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Resistance gene** | **Detection limit**  **(Ct value)** | **AZM patients** | **Non-AZM patients** | ***P* value** |
| ***erm*(A)** | 32.97 | 2.9%  (1/35) | 12%  (2/17) | 0.25 |
| ***erm*(B)** | 34.64 | 83%  (29/35) | 94%  (16/17) | 0.40 |
| ***erm*(C)** | 33.40 | 14%  (5/35) | 5.9%  (1/17) | 0.65 |
| ***erm*(F)** | 31.92 | 54%  (19/35) | 65%  (11/17) | 0.56 |
| ***mef*** | 39.83 | 89%  (31/35) | 94%  (16/17) | >0.99 |
| ***msr*(A)** | 32.56 | 26%  (9/35) | 12%  (2/17) | 0.30 |
| ***msr*(E)** | 36.77 | 60%  (21/35) | 59%  (10/17) | >0.99 |
| ***tet*(M)** | 34.90 | 100%  (35/35) | 100%  (17/17) | >0.99 |
| ***tet*(O)** | 31.01 | 54%  (19/35) | 65%  (11/17) | 0.56 |
| ***tet*(W)** | 33.41 | 94%  (33/35) | 100%  (17/17) | >0.99 |

*P* value determined by Fisher's exact test; AZM: azithromycin.

**Table 3.** Normalised resistance gene abundance in AZM/non-AZM groups

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Resistance gene** | **Patients** | | ***P* value** | **Close contacts** | | ***P* value** |
| **AZM** | **Non-AZM** | **AZM** | **Non-AZM** |
| ***erm*(A)** | 0  (0.0-4.7) | 0  (0.0-0.3) | >0.99 | 0  (0.0-0.0) | 0  (0.0-0.0) | >0.99 |
| ***erm*(B)** | 6.2  (3.6) | 4.9  (2.7) | 0.17 | 4.5  (3.6) | 5.0  (2.9) | 0.61 |
| ***erm*(C)** | 0  (0.0-12.2) | 0  (0.0-6.9) | 0.54 | 0  (0.0-0.0) | 0  (0.0-0.0) | >0.99 |
| ***erm*(F)** | 6.1  (0.0-14.3) | 5.6  (0.0-9.4) | 0.30 | 0  (0.0-12.3) | 3.6  (0.0-9.3) | 0.04 |
| ***mef*** | 3.0  (1.4) | 1.4  (0.9) | <0.0001 | 2.0  (1.6) | 1.3  (1.1) | 0.09 |
| ***msr*(A)** | 0  (0.0-8.8) | 0  (0.0-4.3) | 0.35 | 0  (0.0-7.2) | 0  (0.0-3.8) | 0.79 |
| ***msr*(E)** | 6.6  (0.0-12.9) | 3.9  (0.0-11.6) | 0.75 | 0  (0.0-10.6) | 1.7  (0.0-16.8) | 0.43 |
| ***tet*(M)** | 11.3  (0-15.1) | 10.3  (7.2-12.5) | 0.18 | 10.6  (0-17.9) | 10.4  (4.8-12.7) | 0.35 |
| ***tet*(O)** | 5.5  (0.0-10.2) | 5.3  (0.0-10.3) | 0.91 | 3.7  (0.0-9.7) | 4.2  (0.0-9.6) | 0.78 |
| ***tet*(W)** | 12.8  (0-17.3) | 12.1  (0-15.6) | 0.17 | 10.8  (0-16.1) | 10.4  (0-13.6) | 0.40 |

Resistance gene abundance was normalised to the 16S Ct value of each sample. Data presented as median (min-max), or mean (SD). 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.

**Table 4.** Paired assessment of the resistance gene detection frequency between patients and close contacts

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Resistance gene** | **AZM group** | | **Non-AZM group** | |
| **Odds ratio**  **(95%CI)** | ***P* value** | **Odds ratio**  **(95%CI)** | ***P* value** |
| ***erm*(A)** | N/A | N/A | N/A | N/A |
| ***erm*(B)** | 2.4  (0.3-16.9) | 0.38 | 0  (0.0-0.0) | >0.99 |
| ***erm*(C)** | 0  (0.0-0.0) | >0.99 | N/A | N/A |
| ***erm*(F)** | 16.7  (1.8-152.8) | 0.013 | 4.5  (0.5-41.2) | 0.18 |
| ***mef*** | 3.4  (0.4-28.9) | 0.26 | 0  (0.0-0.0) | >0.99 |
| ***msr*(A)** | 0  (0.0-0.0) | >0.99 | 0  (0.0-0.0) | >0.99 |
| ***msr*(E)** | 0.5  (0.1-2.0) | 0.33 | 2.0  (0.3-14.2) | 0.49 |
| ***tet*(M)** | N/A | N/A | N/A | N/A |
| ***tet*(O)** | 2.2  (0.5-8.6) | 0.27 | 1.8  (0.2-13.2) | 0.59 |
| ***tet*(W)** | 0  (0.0-0.0) | >0.99 | N/A | N/A |

*P* value determined by binary logistic regression; N/A, not applicable, no variance was observed, or the dependent variable has less than two non-missing values, therefore statistics cannot be computed.