**ORIGINAL RESEARCH**

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**TITLE**

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

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**SUMMARY CONFLICT OF INTEREST STATEMENTS**

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

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**ABSTRACT**

**Background:** Long-term macrolide therapy has been shown to provide benefit to those with a range of chronic respiratory conditions. However, there remain concerns about the impact of macrolide exposure on the carriage and abundance of antibiotic resistance genes within the oropharynx. The potential for onward transmission of resistance from macrolide recipients to their close contacts is also poorly understood.

**Research Question:** Does long-term macrolide use impact carriage of resistance within the oropharyngeal microbiota in people with chronic respiratory conditions and risk of onward transmission to their close contacts?

**Study Design and Methods:** Oropharyngeal swabs were collected from 93 individuals with chronic respiratory conditions, of whom 53 were receiving long-term macrolide therapy. An oropharyngeal swab was also collected from a close co-habiting contact of each subject. Detection and abundance of ten macrolide-associated resistance genes with the potential to disseminate via horizontal gene transfer were assessed by quantitative PCR.

**Results:** Detection of resistance genes in macrolide recipients was comparable to that in non-recipients. However, the normalised gene abundance of *erm*(B) was significantly higher in the macrolide recipient group (p=0.045). In the close contacts, no between-group differences in resistance gene detection or abundance were identified. Paired analysis showed that the detection of *erm*(F) and *mef* in macrolide recipients, but not non-recipients, was significantly associated with detection in close contacts (p=0.003 and p=0.004 respectively). Despite the association, the onward transmission risk of these genes was not affected by long-term macrolide therapy (p>0.05 for each gene).

**Interpretation:** In this cohort study, we report increased relative abundance of macrolide resistance genes in those receiving macrolide antibiotics. However, macrolide use was not associated with increased macrolide resistance gene detection rate and there was no evidence that long-term macrolide use increases the onward transmission risk to their close contacts.

**KEY WORDS LIST**

Antibiotic resistance; Asthma; Azithromycin; Cystic fibrosis; Erythromycin; Macrolides; Non-CF bronchiectasis

**ABBREVIATION LIST**

CF: cystic fibrosis;

MNR: macrolide non-recipients;

MNRCC: close contact of a macrolide non-recipient;

MR: macrolide-recipients;

MRCC: close contact of a macrolide recipient;

qPCR: quantitative Polymerase Chain Reaction

**TERMINOLOGIES DEFINED IN THIS STUDY**

Detection: *A qualitative term relating to the presence or absence of a gene based, within the sensitivity limits of the assay used*.

Abundance: *A quantitative term relating to the amount of a gene measured, which has been normalised to the abundance of the 16S rRNA bacterial gene.*

**INTRODUCTION**

Macrolide antibiotics have a range of clinical uses. Antibiotics such as azithromycin, erythromycin, clarithromycin, and roxithromycin, macrolides are used widely in the treatment of specific respiratory1,2 and soft-tissue3 infections. Increasingly, macrolide antibiotics, are also being used in the long-term management of chronic respiratory conditions, including cystic fibrosis,4-6 asthma,7-10 chronic obstructive pulmonary disease,11,12 and bronchiectasis.13-17 Using macrolides in this way appears to be both safe and effective.6,7,14 However, the potential for increased carriage and dissemination of macrolide resistance is a concern both for treatment recipients and for the wider community.17-20

We hypothesised that the carriage of macrolide resistance genes, and non-macrolide resistance genes under co-selection, would be more frequent within the oropharyngeal microbiota of those receiving long-term macrolide therapy for chronic respiratory conditions compared to macrolide-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 co-habiting close contacts of patients, consistent with person-to-person transmission.

We report a quantitative PCR (qPCR)-based assessment of oropharyngeal detection and abundance of 10 macrolide-associated antibiotic resistance genes in 93 individuals with chronic respiratory conditions, of whom 53 were receiving long-term macrolide, and their close contacts.

**STUDY DESIGN AND METHODS**

***Study design and setting***

This is a cross-sectional, single time point cohort study. The study was designed to test whether the detection and abundance of macrolide-associated antibiotic resistance gene in the oropharyngeal microbiota differed between individuals with chronic respiratory conditions who were receiving either long-term macrolide therapy or not (Online Supplement e-Figure 1). In addition, the study was designed to test whether resistance genes detected in individuals with chronic respiratory conditions were detected in close-contacts, and whether long-term macrolide therapy influenced detection in close-contacts. The study was approved by the Mater Health Services Human Resource Ethics Committee (HREC/14/MHS/68) and the Metro South Human Resource Ethics Committee (HREC/15/QPAH/245) in Queensland, Australia. All study participants provided written informed consent.

Participants were approached for recruitment at one of three centres: 1) Mater Hospital South Brisbane, Queensland, Queensland, 2) Greenslopes Private Hospital, Queensland, or 3) Concord Repatriation General Hospital New South Wales. Close contacts were defined as someone who was either a close household contact (spouse, de facto, or family members who has lived with the patient for the preceding 6 months) or was a close family member (parent or sibling or partner) or friend who has had close contact with the patient at least twice per week over the preceding 2 years.

Subjects therefore belonged to one of four groups: 1) macrolide-recipients (MR), defined as those receiving macrolide maintenance therapy (either erythromycin or azithromycin at standard doses for the specific condition) for a chronic respiratory condition; 2) macrolide non-recipients (MNR), defined as those with a chronic respiratory condition who were not receiving macrolide maintenance therapy, and who had not received any macrolide antibiotic in the prior 6 months; 3) close contact of a macrolide recipient (MRCC); or 4) close contact of a macrolide non-recipient (MNRCC). Close contacts were excluded if they had received macrolides in the previous 6 months or had received antibiotics (any class) or hospital treatment in the prior four weeks. Further details of subject inclusion and exclusion criteria are provided in the Online Supplement e-Appendix 1.

***Sample collection***

Collection of oropharyngeal microbiota samples 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*.19 Swabs were stored at -80 °C prior to processing for analysis. Close contact swabs were collected within one week of the index subject (samples were usually collected on the same day).

***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 yield was quantified using a Qubit 2.0 (Invitrogen, Carlsbad, CA, USA).

***Resistance gene detection and quantification***

Total bacterial load was determined using a SYBR Green-based qPCR assay targeting a conserved region of the 16S rRNA gene (Online Supplement e-Table 1). Quantification 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* was achieved using SYBR Green-based assays. Quantification of the *mef*(A)and *mef*(E) macrolide resistance geneswere achieved using a single TaqMan probe-based assay. As this assay measures both *mef* genes, results relating to *mef*(A)and *mef*(E) are referred to as *mef* throughout this manuscript. Oligonucleotides used for each reaction, and amplicon sizes are provided in Online Supplement e-Table 1. 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.20 A detailed description of qPCR methods is provided in Online Supplement e-Appendix 2.

***Statistical analysis***

All data analyses were performed using either R software (version 4.1.0) or GraphPad Prism 9 (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.

Two different binary logistic regression models were employed to assess the propensity for macrolide use to result in transmission of macrolide resistance genes between patients and close contacts. The first, within group comparison, tested the odds of macrolide resistance genes being co-carried between patients and close contacts. The presence or absence of each resistance gene in the patient was the independent variable, while presence or absence in the close contact was the dependent variable. The outcome of this model provided information on the likelihood of macrolide resistance gene co-carriage between macrolide recipients and their close contacts. The second, between group comparison, assessed whether evidence of potential transmission between patients and close contacts (dependent variable) was associated with treatment group (independent variable). Potential transmission was defined as a resistance gene being detected in both patients and their close contacts (1-1), while no evidence of transmission was defined as gene detection in only patients or close (1-0 or 0-1). Instances where neither patients nor their close contacts carried the resistance gene were excluded from the second model as these cases cannot provide evidence of either transmission or no transmission.

**RESULTS**

***Cohort overview***

A total of 149 individuals with chronic respiratory conditions were recruited. Of these, 54 were excluded due to failure to recruit a close contact or to collect all necessary clinical samples and data. A further two patients were excluded due to having received clarithromycin treatment in the 6 months prior (Online Supplement e-Figure 1). Baseline demographic and disease characteristics are shown for both patient and close contact groups in Table 1.

***Macrolide recipients (MR) compared to macrolide non-recipients (MNR)***

The detection (presence/absence) of 10 resistance genes was assessed. Detection rates varied substantially, from 97% detection for *tetM* to 4.3% for *erm*(A) across both MR and MNR groups. No significant differences were identified in the detection frequency of any resistance gene between MR and MNR (Table 2).

The relative abundance (gene levels normalised to the bacterial 16S gene) of the same 10 genes were also assessed. The relative abundance of *erm*(B), a gene what was detected frequently in both groups (MR 89% and MNR 95%), was significantly higher in MR than MNR (p=0.045; Figure 1). However, no significant differences were observed in the relative abundance of any other assessed genes (Table 3).

***Onward transmission: Macrolide recipient close contacts (MRCC) compared to macrolide non-recipients close contacts (MNRCC)***

As a means of investigating onward transmission of macrolide resistance genes, we first compared the detection and abundance of resistance genes between the macrolide recipient close contact (MRCC) and macrolide non-recipients close contacts (MNRCC) (Online Supplement e-Table 2). Similar to MR and MNR groups, detection frequency of macrolide resistance genes in close contacts was variable, ranging from 96% prevalence for *tetM* to 0% for *erm*(A). However, no significant between-group differences in the detection frequency were identified (Online Supplement e-Table 2). The relative abundance of these genes was also compared and were not significantly different between MRCC and MNRCC (Online Supplement e-Table 2).

***Onward transmission: Resistance gene detection between patients and their close contacts***

To assess whether macrolide resistance gene carriage in close contacts was dependent on carriage in their paired patients, within-group, paired analysis was performed. In the macrolide recipient group, detection of *erm*(F) and *mef* in MRCCs was significantly associated with the detection of these genes in their matched MR patient (p=0.003 and p=0.004 respectively, Table 4). This association was not observed for any other genes in the macrolide recipient group, nor for any genes in the macrolide non-recipient group (Table 4). Detection of resistance genes in patients and their close contacts is shown in Online Supplement e-Figure 2.

Despite the association of *erm*(F) and *mef* detection between MRCC and MR patients, the relative abundances of these genes were significantly lower in close contacts compared to corresponding MR patients (Figure 2).

***Onward transmission: Paired resistance gene detection between macrolide recipient and macrolide non-recipient groups***

To further investigate the impact of long-term macrolide therapy on macrolide resistance gene transmission, we compared the risks of patient-close contact macrolide resistance gene transmission between groups (macrolide recipients vs macrolide non-recipients) using our second binary logistic regression model (details see statistical analysis section). The risk of a patient and close contact both carrying a macrolide resistance gene was not significantly higher in macrolide recipients and macrolide non-recipients (p>0.05, Table 5). In other words, none of the resistance genes assessed were more likely to be detected in the close contacts of the macrolide recipient group compared to the close contacts of the macrolide non-recipient group.

**DISCUSSION**

1. Q4 from R1: Highlight the potential for these relationships to influence AMR transmission in the discussion
2. Q2 from R2: A new discussion of our transmission risk finding

*“We do not show that macrolide use was associated with co-detection of resistance genes, indicating an absence of macrolide-associated onward transmission. Had we identified evidence that macrolide use was associated with co-detection transmission between patients and close contacts, further studies would be needed to support any conclusion that onward transmission occurred. This is because co-detection does not equate transmission. Such studies would necessitate longitudinal studies, with precise methods to detect signatures of transmission, such as strain typing from cultured isolates. However, a lack of co-detection does strongly reflect a lack of onward transmission and support the conclusions of this study.”*

1. Q3 from R2: Highlight the considerations on why we included highly prevalent macrolide and tetracycline genes as assessment target in this study
2. Q5 from R2: Highlight another point why we detect tetracycline genes: macrolide exposure can co-select tetracycline resistance genes via MGE
3. Q6 from R2: Importance of resistance transmission in the gut and the rationale of our decision to measure the impact of macrolide therapy on carriage and co-detection of resistance within the oropharynx

There is a concern that an increased use of macrolide antibiotics in the clinical management of common chronic respiratory diseases could contribute to the growing population-level burden of antibiotic resistance carriage. Our aim was therefore to investigate the impact of long-term macrolide treatment on the carriage of resistance determinants within the oropharyngeal microbiota in patients with chronic respiratory conditions, and potential onward transmission to close contacts. Our cohort consisted of adults with asthma, CF and bronchiectasis, conditions in which long-term macrolide therapy has been shown to confer substantial benefit.4-7,13-15

The resistance genes assessed here have been demonstrated to be common determinants of macrolide susceptibility in respiratory bacterial pathogens or to increase in oropharyngeal prevalence with long-term macrolide exposure.19,21 Seven of the assessed genes confer macrolide resistance via 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*, *msr*(A) and *msr*(E)), and are all associated with mobile genetic elements.22-27 Three tetracycline resistance genes: *tetM*, *tetO*, and *tetW* that are associated with macrolide exposure,19,20 and which can be co-located on mobile genetic elements with macrolide resistance genes,20,22,28,29 were also assessed.

Our investigation highlighted how commonly many of the assessed determinants are carried, both in patients being treated for chronic respiratory conditions and in non-recipients of macrolides. 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.19 Indeed, we detected *mef* in 63% of macrolide non-recipient close contacts. We also detected *erm*(B) and *tetM* in a high proportion of macrolide non-recipient close contacts (85%, and 95%, respectively), again, in keeping with the high relative prevalence of these genes in oropharyngeal streptococci, reported previously to be 30% and 40%, respectively.19 Such high frequency of detection might reflect their common presence on mobile genetic elements, including conjugative transposons and conjugative plasmids,30,31 that can move readily between bacterial species.30,31

Interestingly, we did identify a significant increase in the relative abundance of *erm*(B) in macrolide recipients compared to non-recipients, supporting the previous data with the increased abundance of *erm*(B) found in the oropharynx of patients with bronchiectasis after 48 weeks of erythromycin therapy.21 However, the absence of substantial differences in gene carriage frequency, either between macrolide recipient and non-recipient groups, or between patient groups and their respective close contacts, suggests that, despite the difference in the relative abundance of *erm*(B), chronic macrolide exposure is not associated with noticeably increased carriage frequency of macrolide-associated resistance genes. Several common oropharyngeal bacterial genus have been found to carry *erm*(B), including *Streptococcus*,32,33 *Haemophilus*,34 *Gemella*,35,36 *Staphylococcus*,35,37 *Enterococcus*,35 *Neisseria*,38 and *Campylobacter*.39 Higher levels of these bacteria that carried *erm*(B) may potentially increase the likelihood of onward transmission, with the risk of such transmission greatest for those living in close physical proximity. However, our comparison of resistance genes frequency between the two close contact groups did not show any evidence of increased resistance gene carriage in macrolide recipient close contacts and is therefore not likely to be of importance.

Nonetheless, to explore the potential risk for onward transmission, we undertook three analyses. Firstly, by comparing close contacts of macrolide recipients and non-recipients, we found that both groups had comparable levels of resistance gene carriage, indicating no increased resistance gene carriage in close contacts of patients receiving macrolides. Secondly, by comparing detection rates within groups, we identified that carrying *erm*(F) or *mef* (not *erm*B as above) by close contacts of macrolide recipients was significantly associated with carriage in their paired patient. Such an association was not observed in macrolide non-recipients, indicating potential onward transmission.The *erm*(F) and *mef* genes are located on transmissible elements found in *Prevotella* spp. and *Streptococcus* spp. Respectively.40-42 Macrolide-resistant streptococci are of clinical concern as macrolides are a common single-agent antibiotic for streptococcal infections in patients with penicillin allergies.43 However, subsequent analysis comparing transmission risk between macrolide recipient and non-recipient groups found that macrolide use was not predictive of co-carriage. Taking all three analyses together, our findings indicate that, while *erm*(F) and *mef* had a significant likelihood of co-carriage between macrolide recipients and their close contacts, there was no indication that close contacts of macrolide recipients had a higher carriage compared to close contacts of macrolide non-recipients, or that macrolide use was significantly associated with this effect.

Our study did have limitations and considerations that should be noted. Macrolide recipient and non-recipient groups differed in their clinical characteristics and treatment exposures, including the former having a higher frequency of hospital admission and a greater burden of acute non-macrolide antibiotics. However, these disparities were not found to influence oropharyngeal carriage of macrolide resistance determinants directly in our study. Second, both patients and close contacts were stable at the time of sample collection, limiting the risk of transmission of resistant bacterial populations compared to periods of exacerbation. For example, periods of exacerbation can include increased coughing,44 which has been shown to promote dissemination of potentially resistant bacteria through the production of cough aerosols.45,46 Further, during periods of stability, intact commensal microbial systems, including those in the oropharynx, resist colonisation by external bacterial populations.47 This protection is greatly reduced when microbiota are disrupted, such as during respiratory viral infection.48 The risks of onward transmission of resistant bacterial populations might therefore vary according to the health of both macrolide recipients and close contacts.

Analysis was based on resistance gene carriage; it did not include assessment of phenotypic resistance. This analysis also did not determine whether the resistance gene carriage was primarily occurring in commensal bacteria or pathogens. 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. Resistance conferred by point mutations or amino acid alteration in ribosomal proteins, two common macrolide resistance mechanisms for certain pathogens,49,50 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-recipient group, a difference that reflected a lower rate of close contact recruitment in non-recipients.

In summary, we report that detection of macrolide and macrolide-associated resistance genes is common in the oropharyngeal microbiota, irrespective of long-term macrolide use. In keeping with previous studies, increased abundance of the *erm*(B) gene in patients was associated with long-term macrolide use. Finally, while some resistance genes were significantly co-carried between patients and close contacts in the macrolide recipient group, macrolide use was not associated with increased risk of carriage in close contacts.

**INTERPRETATION**

This is the first cross-sectional cohort study that report the impact of long-term macrolide therapy on oropharyngeal macrolide resistance gene carriage in healthy close contacts of people with chronic lung diseases. Given long-term macrolide therapy was not associated with increased risk of acquiring macrolide resistance genes in the oropharynx of close contacts, our study provides new evidence to further support existing guidelines for the use of long-term macrolide therapy in people with chronic lung diseases.

**TAKE-HOME POINT**

**Study Question:** Does long-term macrolide therapy impact carriage of macrolide resistance in patients with chronic respiratory conditions and does it increase the risk of onward transmission to close contacts?

**Results:** Macrolide resistance genes were higher in patients receiving long-term macrolide therapy; however, there was no evidence that long-term macrolide use increases the onward transmission risk to their close contacts.

**Interpretation:** This study provides new evidence to further support existing guidelines for the use of long-term macrolide therapy in people with chronic lung diseases.

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**Guarantor:** Dr. Steven Taylor is the author responsible for the content of the manuscript.

**Author’s Contributions:** The study was conceived by GBR and LDB. LDB, LM, RT, 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.

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**Table 1.** Study population characteristics

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Characteristic** | **MR** | **MNR** | ***P* value** | **MRCC** | **MNRCC** | ***P* value** |
| Sample size (N) | 53 | 40 |  | 53 | 40 |  |
| Age, years\* |  |  |  |  |  |  |
| Median (95% CI) | 41 (30-53) | 60 (34-73) | 0.25 | 53 (47-58) | 62 (49-69) | 0.54 |
| Sex, n (%)† |  |  |  |  |  |  |
| Male | 27 (51) | 18 (45) | 0.68 | 22 (42) | 18 (45) | 0.83 |
| Female | 26 (49) | 22 (55) | 31 (58) | 22 (55) |
| Respiratory condition, n (%) |  |  |  |  |  |  |
| Cystic fibrosis | 35 (66) | 10 (25) | 0.0001 | 0 | 0 | >0.99 |
| Asthma | 0 | 7 (18) | 0.002 | 5 (9) | 6 (15) | 0.52 |
| Non-CF Bronchiectasis | 18 (34) | 23 (58) | 0.035 | 0 | 0 | >0.99 |
| Smoking status, n (%)† |  |  |  |  |  |  |
| Non-smoker | 44 (83) | 32 (80) | 0.79 | 35 (66) | 21 (53) | 0.21 |
| Current smoker | 0 | 0 | >0.99 | 2 (4) | 3 (7) | 0.65 |
| Ex-smoker | 8 (15) | 8 (20) | 0.59 | 15 (28) | 16 (40) | 0.27 |
| Not provided | 1 (2) | 0 | >0.99 | 1 (2) | 0 | >0.99 |
| Hospital admission in last 4 weeks, n (%)† | 6 (11) | 2 (5) | 0.46 | 0 | 0 | >0.99 |
| Macrolide exposure, n (%)† |  |  |  |  |  |  |
| Azithromycin | 40 (75) | 0 | <0.0001 | 0 | 0 | >0.99 |
| Erythromycin | 13 (25) | 0 | 0.0004 | 0 | 0 | >0.99 |

Abbreviations: MR: patients who were receiving long-term macrolide therapy; MNR: patients who were not receiving any macrolide therapy. MRCC: close contacts of patients who were receiving long-term macrolide therapy; MNRCC close contacts of patients who were not receiving any macrolide therapy. \*Median (95% CI), Mann-Whitney U test; †n (%), Fisher's exact test. Respiratory condition categorized by primary diagnosis.

**Table 2.** Resistance gene detection frequency in patients stratified by macrolide use.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Resistance gene** | **MR** | | **MNR** | ***P* values** |
| *erm*(A) | 3.8%  (2/53) | 5.0%  (2/40) | | 0.99 |
| *erm*(B) | 89%  (47/53) | 95%  (38/40) | | 0.46 |
| *erm*(C) | 19%  (10/53) | 13%  (5/40) | | 0.57 |
| *erm*(F) | 68%  (36/53) | 78%  (31/40) | | 0.36 |
| *mef* | 74%  (39/53) | 83%  (33/40) | | 0.33 |
| *msr*(A) | 36%  (19/53) | 25%  (10/40) | | 0.37 |
| *msr*(E) | 66%  (35/53) | 73%  (29/40) | | 0.65 |
| *tetM* | 94%  (50/53) | 100%  (40/40) | | 0.26 |
| *tetO* | 64%  (34/53) | 78%  (31/40) | | 0.18 |
| *tetW* | 85%  (45/53) | 98%  (39/40) | | 0.07 |

Abbreviations: MR: patients who were receiving long-term macrolide therapy; MNR patients who were not receiving any macrolide therapy. *P* value determined by Fisher's exact test.

**Table 3.** Normalised resistance gene abundance in patients stratified by macrolide use.

|  |  |  |  |
| --- | --- | --- | --- |
| **Resistance gene** | **MR** | **MNR** | ***P* values** |
| *erm*(A) | 0  (0.0-10.5) | 0  (0.0-7.1) | 0.39 |
| *erm*(B) | 7.5  (0.0-12.4) | 6.9  (0.0-10.8) | 0.045\* |
| *erm*(C) | 0  (0.0-13.2) | 0  (0.0-8.0) | 0.14 |
| *erm*(F) | 7.6  (0.0-12.4) | 6.2  (0.0-11.9) | 0.22 |
| *mef* | 4.4  (0.0-6.7) | 3.9  (0.0-7.5) | 0.20 |
| *msr*(A) | 0  (0.0-13.2) | 0  (0.0-9.1) | 0.15 |
| *msr*(E) | 7.3  (0.0-13.0) | 5.7  (0.0-15.9) | 0.07 |
| *tetM* | 5.9  (0.0-8.9) | 5.5  (2.1-7.6) | 0.42 |
| *tetO* | 6.4  (0.0-10.5) | 6.3  (0.0-12.3) | 0.39 |
| *tetW* | 5.2  (2.7) | 4.8  (2.2) | 0.46 |

Abbreviations: MR: patients who were receiving long-term macrolide therapy; MNR patients who were not receiving any macrolide therapy. Resistance gene abundance was normalised to the 16S Ct value of each sample. Data presented as median (min-max) or mean (SD) depending on data distribution. *P* values were determined by Mann-Whitney U test or Student’s t test with Welch's correction. \* *P* value is not significant after FDR correction

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Resistance gene** | **MR vs MRCC** | | **MNR vs MNRCC** | |
| **Odds ratio**  **(95% CI)** | ***P* value** | **Odds ratio**  **(95% CI)** | ***P* value** |
| *erm*(B) | 3.4  (0.5-22.9) | 0.21 | 1.3×10-7  (0-Inf) | >0.99 |
| *erm*(C) | 5.1  (0.6-41.9) | 0.13 | 8.5  (0.4-163.9) | 0.16 |
| *erm*(F) | 11.8  (2.3-59.6) | 0.0029 | 1.7  (0.4-7.6) | 0.50 |
| *mef* | 7.3  (1.9-28.4) | 0.0044 | 1.3  (0.3-6.9) | 0.75 |
| *msr*(A) | 1.5  (0.5-4.9) | 0.48 | 1.8  (0.4-8.2) | 0.43 |
| *msr*(E) | 0.8  (0.3-2.7) | 0.74 | 1.1  (0.3-4.5) | 0.87 |
| *tetM* | 2.1×10-7  (0-Inf) | >0.99 | N/A | N/A |
| *tetO* | 2.7  (0.8-8.5) | 0.099 | 1.7  (0.4-7.6) | 0.50 |
| *tetW* | 2.4  (0.5-12.0) | 0.29 | 2.5×10-7  (0-Inf) | >0.99 |

Abbreviations: MR: patients who were receiving long-term macrolide therapy; MNR patients who were not receiving any macrolide therapy. CC: close contacts. *P* value determined by binary logistic regression. Analyses could not be performed for *tetM* of the macrolide non-recipient group and for *erm*(A) (both groups) due to no variance between groups.

**Table 5.** Assessment of long-term macrolide use on onward transmission risk of macrolide resistance genes.

|  |  |  |
| --- | --- | --- |
| **Resistance**  **gene** | **Macrolide recipient group vs Macrolide non-recipient group** | |
| **Odds ratio**  **(95% CI)** | ***P* value** |
| *erm*(A) | 1.0  (0-Inf) | >0.99 |
| *erm*(B) | 1.0  (0.4-2.9) | 0.96 |
| *erm*(C) | 1.0  (0.07-13.9) | >0.99 |
| *erm*(F) | 1.0  (0.4-2.5) | 0.97 |
| *mef* | 1.6  (0.6-3.9) | 0.33 |
| *msr*(A) | 1.3  (0.3-5.0) | 0.73 |
| *msr*(E) | 0.6  (0.2-1.5) | 0.25 |
| *tetM* | 0.5  (0.09-2.7) | 0.43 |
| *tetO* | 0.9  (0.4-2.2) | 0.82 |
| *tetW* | 0.7  (0.3-2.0) | 0.55 |

*P* value determined by binary logistic regression. Pairs with resistance genes being undetected in both patients and the close contacts are excluded from the analysis as the presence of those pairs in the analysis do not provide any clinical transmission information and will impact the accuracy of the results.

**FIGURE LEGENDS**

**Figure 1. Comparison of *erm*(B) abundance in macrolide recipients and macrolide non-recipients.**

*erm*(B) abundance normalised to bacterial 16S copies. Bars show mean and standard deviation; significance calculated by Mann-Whitney U test. \*p<0.05

**Figure 2. Comparison of *erm*(F) and *mef* abundance between patient/close contact pairs.**

Gene levels normalised to bacterial 16S copies. Significance of gene abundance calculated by Wilcoxon matched-pairs signed rank test.