**CLINICAL STUDY PROTOCOL**

Macrolide-resistance in the oropharyngeal flora of cohabitants of non-CF bronchiectasis patients treated with long-term erythromycin or azithromycin. A cross sectional cohort study.

**- Research Proposal-**

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**Ethics Submission**

**Title**

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**Background**

Non-CF bronchiectasis is responsible for significant morbidity and mortality and represents a significant clinical burden to both patients and healthcare systems as a whole. Hospitilizations and deaths attributed to bronchiectasis in developed countries including Australia have steadily increased in recent years.1,2. Bronchiectasis is also responsible for longer hospital admissions, more frequent outpatient visits, and greater treatment burden than matched controls with other chronic conditions.3.

Traditionally, outpatient pharmacological management of non-CF bronchiectasis has been limited. Fortunately, multiple recent prospective randomized controlled trials and a maeta-analysis have demonstrated a role for long term macrolide antibiotic therapy in a selected subgroup of patients.4,5,6. Benefits include a reduction in exacerbation frequency, improved quality of life, improved symptoms, and improved lung function. Despite these encouraging results, the predominant concern for long-term macrolide therapy is an increased development of macrolide resistance in pneumococcal isolates which was repeatedly demonstrated in the above trials. The choice of macrolide is also significant with evidence to suggest that the longer acting azithromycin use may confer high rates of macrolide resistance than erythromycin7.

While direct induction of antimicrobial resistance in patients prescribed long term macrolide therapy is a legitimate concern, of potentially greater concern is the potential impact such therapy has on population resistance rates. Background rates of macrolide resistance in *Streptococcus pneumoniae* isolates vary significantly between populations however most countries report rates of at least 10%, rising to almost 100% in some regions7. A meta-analysis in 2005 including over 400 studies reported pooled macrolide resistance rates of approximately 30% in *Streptococcus species8*.

Macrolide resistance has been shown to occur in tandem with the development of penicillin resistance. In addition macrolide resistance can occur independently of this and in some parts of the world macrolide resistance rates exceed those of penicillin resistance9. Multiple population studies have demonstrated an association between macrolide exposure and resistance rates with increased macrolide use leading to an increase in population resistance7.

An important mechanism for the development of population resistance is clonal spread with nasopharyngeal carriage responsible for dissemination of resistant strains from carriers to close contacts10. Clonal spread of resistant strains may be facilitated by hospitals, chronic health care facilities, and day care centres. Transmission is also accelerated via selection pressure from antibiotic use with recent antibiotic use associated with higher rates of nasopharyngeal carriage of macrolide resistant *S. pneumoniae* isolates11.

Macrolides therapy now serves a vital role in the long-term management of multiple chronic lung diseases including CF bronchiectasis, non-CF bronchiectasis and chronic obstructive pulmonary disease (COPD). Concerns over the development of population resistance are valid given the association between the magnitude of macrolide use and resistance rates. Although clonal spread of resistance via nasopharyngeal carriage is thought to be a mechanism for the spread of resistance, the exact rates of clonal transmission have not been fully explored, particularly in non-CF bronchiectasis. Our study will attempt to provide an answer to this question. The results of this study will add to the literature to guide informed and rationale prescription of long-term macrolide therapy in non-CF bronchiectasis.

**Aim**

To quantify macrolide resistance in oropharyngeal streptococcal species in the cohabitants of patients prescribed long-term erythromycin or azithromycin for non-CF bronchiectasis, compared with matched, macrolide-naïve controls.

**Objectives**

1. To determine if the cohabitants of subjects taking maintenance erythromycin or azithromycin therapy will harbor macrolide resistant oropharyngeal flora in greater proportions than close contacts of control subjects not taking macrolide antibiotics
2. To characterise oropharyngeal microbiota composition of non-CF bronchiectasis subjects taking maintenance erythromycin or azithromycin therapy, their cohabitants, and matched subjects not prescribed macrolides

**Hypothesis**

Cohabitants of non-CF bronchiectasis subjects who have received long-term maintenance erythromycin or azithromycin will harbor macrolide-resistant oropharyngeal streptococci in higher proportions than the cohabitants of matched control subjects not exposed to macrolide therapy.

**Design**

The study will be a prospective, cross sectional case controlled cohort study.

**Setting/Location**

This will be a multi-centre study carried out in the sites listed below:

1. Department of Respiratory Medicine, Mater Health Services, South Brisbane, QLD, Australia.
2. Gallipoli Medical Research Centre, Greenslopes Private Hospital
3. Concord Hospital
4. SAHMRI

**Study Population**

Patients to be included in the study will be screened from:

1. Patients attending Respiratory Medicine outpatient clinics and private consulting rooms.
2. Pre-existing clinical and research databases.

Patients screened will need to meet the below inclusion and exclusion criteria.

The following medications will be permitted (in both cases and control cases, but not cohabitants), provided they represent a regular maintenance therapy that has been continuous for at least the preceding 3 months:

* Inhaled/ nebulized antibiotics in stable daily dose
* Inhaled corticosteroids in stable, daily dose
* Inhaled hypertonic saline or mannitol in stable daily dose
* Inhaled bronchodilators of any class, in stable daily dose

1. **Index cases (patients prescribed erythromycin or azithromycin)**

Inclusion criteria

* Able to give informed consent
* Age 18 and above
* Established diagnosis of non-CF bronchiectasis according to standard criteria
* Prescribed erythromycin or azithromycin daily for at least the preceding 6 months, with assurance of past compliance from patient.
* Has lived with at least 1 other person for the preceding 6 months.
* Clinically stable for preceding 4 weeks.
* (Cohabitants must meet separate inclusion/exclusion criteria outlined below).

Exclusion criteria

* Cystic fibrosis
* Use of supplemental oral or parenteral antibiotics (of any antibiotic class) in the preceding 4 weeks (other than maintenance erythromycin or azithromycin).
* Hospital admission in previous 4 weeks.
* Living alone with no close contacts/cohabitants
* Current smokers

1. **Control cases (subjects not taking maintenance erythromycin or azithromycin)**

Inclusion criteria

* Able to give informed consent
* Age 18 and above
* Established diagnosis of non-CF bronchiectasis according to standard criteria
* No macrolide antibiotic use in the prior 6 months, and must not have received any azithromycin in the prior 12 months
* Has lived with at least 1 other person for the preceding 6 months.
* Clinically stable for preceding 4 weeks.
* (Cohabitants must meet separate inclusion/exclusion criteria outlined below).

Exclusion criteria

* Cystic fibrosis
* Use of any macrolide in the prior 6 months, or of azithromycin in the prior 12 months
* Use of supplemental non-macrolide oral or parenteral antibiotics in the preceding 4 weeks
* Hospital admission in previous 4 weeks
* Living alone with no close contacts/cohabitants
* Current smokers

1. **Cohabitants (of either index or control cases)**

Inclusion criteria

* Domiciled in the same residence as the case or control case, for at least the prior 6 months
* Considered to be a ‘close’ contact or cohabitant. This would include spouse or defacto, or family members. If more than one cohabitant, then the one with the closest contact (ie mouth to mouth contact – kissing), will be selected.
* No chronic respiratory disease, excepting well-controlled asthma (such subjects may be prescribed inhaled bronchodilators of any class, and/ or inhaled corticosteroids provided it is in low-dose, no more than 500 mcg/ day beclomethasone dose-equivalent)
* No antibiotic use (any class) in the prior 3 months
* No hospital admission in preceding 6 months
* No macrolide use in the prior 6 months, and no azithromycin use in the prior 12 months

Exclusion criteria

* A cohabitant who does not have close contact with the case; eg boarders or lodgers would not be suitable.
* A chronic respiratory disease other than well-controlled asthma
* Consumption of any antibiotic in the prior 3 months, any macrolide in the prior 6 months, azithromycin in the prior 12 months

**Study Procedure**

In those subjects meeting the above criteria the following will be carried out:

Subjects will be provided with information sheets at the time of recruitment and will need to give written consent to be involved prior to collection of swabs. A standardized consent form will be used.

Oropharyngeal swabs will be taken either during outpatient clinic appointments or on an appointment basis at a time suitable for those involved.

The swab process will consist of:

* Two oropharyngeal swabs of each patient and their cohabitant in the study group
  + One FLOQSwab (dry swab)
  + One Aerobic Culture Swab
* Two oropharyngeal swabs (as above) of each patient and their cohabitant in the control group.

Swab technique: Samples of oropharyngeal flora will be obtained by means of swab pressed over the tonsils and posterior pharyngeal wall, avoiding the jaws teeth and gingiva on withdrawal12.

An effort will be made to take swabs from patients and their cohabitants at the same time point if possible but may occur at different time points.

Swabs will be taken by staff members involved in the study.

A record of subject involvement will be taken with a standardized data collection tool and stored electronically, and will include the following

* Age
* Gender
* Medical Conditions
* Medication history including; antibiotic exposure in the last 12 months, use of inhaled corticosteroids, use of nebulised antibiotics; any nebulised/inhaled therapies
* Recent hospitalizations
* Degree of contact between close contacts and subjects(ie whether the contact is a spouse, sibling or child etc) and the time period in which the contact occurred will be documented.
* Vaccination history
* Smoking History

*Oropharyngeal swab processing and data analyses*

Aerobic culture oropharyngeal swabs will be placed in an aerobic medium containing skimmed milk, glucose and glycerol adapted from Gibson and Khoury and stored at -80 degrees celsius until further analysis12.FLOQSwabs will be stored directly at -80oC. Samples will be stored in the respective departments and batch sent for further processing.

All subsequent sample processing will be performed at the South Australian Health and Medical Research Institute, Adelaide (under the supervision of A/ Prof Geraint Rogers).

Oropharyngeal swabs will subsequently be processed for each of the following outcome measures:

*Oropharyngeal streptococcal culture and sensitivity testing according to methods previously described12:*

The samples will be thawed and, vortexed and inoculated on Columbia CAN agar (Beckton Dickinson) with or without erythromycin (2micrograms/ml) using a sterile spreader. Plates will be incubated at 37 degrees celsius in 5% CO2/95% air. Bacterial colonies will be assessed on both erythromycin treated plates and non-antibiotic plates to determine the rate of macrolide resistant bacteria. Media quality control will be performed to ensure growth of only gram-positive organisms with sterility assessment at 48 hours.

Proportional macrolide resistance will be compared between index-cohabitants and control-cohabitants using tests according to normality of distribution of the data. Previously these data have been non-normally distributed12 and it is therefore likely that rates of proportional macrolide resistance will be compared by Mann-Whitney U test.

*Quantification of bacterial resistance genes*

Multiplex endpoint PCR assay will be used to determine the presence of a panel of resistance genes (erm(A), erm(B), mef, tet(M), tet(O), tet(K), tet(L) as described in Malhotra-Kumar13.Where resistant genes are detected; gene specific qPCR assays will be used to determine their abundance relative to total bacterial load. This will be determined by 16srRNA gene qPCR as previously described14. Differences in relative abundance of bacterial resistance genes will be compared between index case-cohabitants and control-cohabitants by Mann-Whitney U-test.

*Microbiota composition analysis*

Oropharyngeal swabs will undergo DNA extraction, followed by paired-read 16S-rRNA gene V1-V3 amplicon sequencing, using the illumine MISeq platform as previously described14.

Subsequent data analyses will utilize the following principles: Bray Curtis dissimilarity matrices will be generated based on microbiota profiles from visit 1, 2 and 3 samples. Distribution of microbiota similarity will be visualized by nonmetric multidimensional scaling (NMS) and the existence of significant between group differences tested by PERMANOVA test. Specifically assessment will be made on whether significant differences exist in microbiota composition between azithromycin or erythromycin groups at each timepoint. Where significant variances occur, SIMPER analysis will be used to determine the contribution to observed subgroup differences in microbiota composition by determining individual bacterial taxa as preciously described14,15. Significance of these taxon-level differences will be tested based on relative abundance variation (Mann Whitney U Test).

*Direct detection and quantification of organisms*

Streptococcus Pneumoniae, Mycoplasma Pneumoniae, Bordatella Pertussis will be determined using qPCR assays targeting lytA, 23s rRNA spacer region and IS481 region respectively as previously described16. Differences in levels of the above bacterial groups will be tested for significance using the Mann Whitney U Test.

**Statisical analysis, sample size, power.**

Based upon the mean 44% increase in oropharyngeal macrolide resistance seen after 1 month of erythromycin therapy in the BLESS study, baseline SD of 18%12 and assuming incomplete (estimated 25%) transmission of macrolide resistance from index cases to cohabitants, 44 cohabitants in each arm will provide 80% power at the 5% significance level to demonstrate an 11% difference in proportional macrolide resistance rates between case-contacts and control-contacts. Numbers have been rounded up to 50 in each group to account for subject and sample attrition.

**Study duration and timeline**

Aug 2016 – submission of updated protocol and NEAF to Metro South HREC.

Nov 2016 – approval of ethics submission.

Dec 2016 – approval of governance for study.

Jan 2016 – commence recruitment of patients and collection of swabs.

January 2018 – completion of patient recruitment and collection of swabs.

February 2019 – data analysis and assessment of results.

**Data Management and Storage**

* Data will be collected by staff involved in the study. This may include medical officers, clinical nurses, pathology staff, and research nurses.
* A standardized data collection tool in written form will be used to record patient involvement and the collection of swabs at the time of recruitment.
* The above data will then be transferred onto an electronic data collection program.
* Data in paper form will be stored securely in a locked office of the researcher.
* Electronic data will be stored on a password protected computer.
* Collection and use of individually identifiable data with the individual’s consent. Data will be individually identifiable to enable efficient recruitment and adequate follow-up.

**Resource implications**

Sample collection and storage will be undertaken with existing research staff and using existing research storage facilities. Sample processing and analyses will be performed at SAHMRI under the supervision of our close collaborator A/Prof Geraint Rogers.

**Financial obligations/ compensation of participants**

There will be no direct financial obligations to the subject for involvement in the current study. Patients will receive compensation for parking and offered compensation for public transport. There is no additional financial compensation or incentive for involvement in this clinical trial.

**Potential risks**

As there is no therapeutic or pharmacological intervention involved in this study, there is no quantifiable risk to the subjects physical safety.

The physical action of taking oropharyngeal swabs poses negligible risk to the subjects.

**Protection against risks**

All procedures will be performed by an experienced clinical or research staff member.

Care will be taken to ensure that any potential for patients to pressure close contacts into participating is minimized. There is no significant incentive for patients or their close contacts stand to benefit directly from participation in the study, either from data and sample collection, or in the form of rewards or incentives from the study. In addition there are no negative outcomes to the patient if they chose not to be involved or if their close contacts are not recruited. This removes any incentive for the patient to pressure their contacts into participation. Researches will discuss the study with patients and reiterate that participation is voluntary for themselves and their close contacts. An introduction sheet (attached) will be provided to close contacts as an introduction to the role of the study itself with no pressure to participate. Close contacts will meet with researchers alone in a confidential setting without the patient present in order to discuss the study and ask questions, once they have expressed an interest to participate. Researchers will reiterate in discussions, as in all documentation, that all involvement is voluntary and that they may withdraw at any time.

**Adverse Events**

Adverse events will be documented as they occur in the participant file.

**Confidentiality**

The research will involve collection of data, however participants will not be identifiable from the data recorded.

Data will be held only by the investigators undertaking the trial, and participants will be assigned an identification code that will not be decipherable by other persons. Future publications arising from this work will not identify individual participants. Data with participant identifiers will not be released.

**Informed consent**

Participants in this study will be provided with written material detailing the trial, its conduct and potential risks and benefits of the study. This information will be personally provided by the study investigator, who will also explain the details regarding this, answer any questions, and obtain informed consent. Participants will not have any time-limit placed upon them regarding their decision to participate in the trial.

Participants who may potentially be involved in the study will be approached either by telephone or during the course of their usual clinical review. Those interested will be given an information sheet providing the details of the trial, to read at their leisure, to enable them to make any decision in their own time.

The potential participant will be encouraged to clarify any queries they may have with the principal investigator. Discussions with potential subjects will enable determination of subjects who may not fully comprehend the study.

The subjects will be mentally and physically competent to give informed consent or the study.

**Other ethical implications**

I am not aware of any other ethical issues pertaining to this study.

**Potential benefits of the study**

This study has the potential to demonstrate a relationship between the development of macrolide resistance in individuals treated with maintenance erythromycin or azithromycin and their close contacts. Such a finding would provide substantiation for the spread of close community resistance from the use of macrolide in individuals on maintenance therapy.

This finding has significant implications for the way that macrolides are used in medical practice in the future and would provide direct evidence for the mechanism of community resistance arising in subjects not directly antibiotic exposed.

**Therapeutic alternatives**

**Therapeutic alternatives**

N/A – this is not an interventional/ therapeutic study.

**Risk/ benefit relationship**

The risks related to this trial are anticipated to be minimal, and therefore it is expected that the benefits related to salient academic evidence regarding the use of macrolides in non-CF bronchiectasis and chronic respiratory disease are greater. The current research will help inform an appropriate management strategy with regards to the use of erythromycin in bronchiectasis and chronic respiratory disease, as well as generating data that will allow a more comprehensive understanding of the risk/ benefit relationship of their use in bronchiectasis and chronic respiratory disease subjects.

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