IMPAART STUDY PROTOCOL

A study of the **Im**pact of **P**enicillin **A**llergy on **A**ntimicrobial **R**esistance and ou**T**comes

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Contents

1. BACKGROUND AND RATIONALE

Effective and safe antibiotic prescribing for the treatment of infections is obstructed by incorrect penicillin allergy records for an estimated 2.7 million people in England (West et al., 2019). In the UK, 6% of primary care and 14% of hospitalised patients are reported to have a penicillin allergy, but only a fraction of these patients have genuine allergy on further testing (Baxter et al., 2020, Shenoy et al., 2019, West et al., 2019). Patients who have a penicillin allergy record have a higher prevalence of antimicrobial resistance (AMR), have longer hospital stays, receive repeated courses of antibiotic therapy and have higher rates of in-hospital mortality when compared to patients without a penicillin allergy record (Macy and Contreras, 2014, Blumenthal et al., 2018, West et al., 2019). These patients are also more likely to receive broad spectrum antibiotics such as macrolides, tetracyclines, cephalosporins, fluroquinolones and clindamycin (West et al., 2019).

Escalating antimicrobial resistance (AMR) is a global health concern, to which penicillin allergy contributes. In the context of the current global COVID-19 pandemic tackling AMR must be considered alongside managing the pandemic. Antibiotic use in patients with COVID-19 is high, with three quarters of inpatients being prescribed antibiotics (Langford et al., 2021) despite the prevalence of bacterial infections being reported as 7-9% (Langford et al., 2020, Lansbury et al., 2020, Rawson et al., 2020). The effect of the pandemic on AMR is still uncertain, however disproportionate and unnecessary antibiotic prescribing in patients hospitalised with COVID-19 is likely to impact on AMR and on patient outcomes. A penicillin allergy record will influence antibiotic prescription and patients with a penicillin allergy record are more likely to receive broad spectrum antibiotics in place of recommended first line penicillin antibiotics when compared with patients without a penicillin allergy. The impact of antibiotic prescribing in patients with COVID-19 who have a penicillin allergy has not been investigated. However, if we extrapolate from pre-pandemic data, we can assume that patients hospitalised with COVID-19 who have a penicillin allergy record and are treated for bacterial infections will be at risk of longer hospital stays, higher rates of treatment failure and have higher mortality when compared to patients without a penicillin allergy record.

The focus of this research is the intersection between COVID-19, penicillin allergy and AMR. Penicillin allergy records are well recognised to have a significant impact on antibiotic prescribing, clinical outcomes and AMR. We will explore how antibiotic prescribing and clinical outcomes are impacted by penicillin allergy in patients with COVID-19, how penicillin allergy affects the oral and GI resistome (the composition of AMR genes present), and whether removing incorrect penicillin allergy records results in a change in the composition of the oral and GI resistome.

Studies analysing the oral resistome (composition of AMR genes found in the oral microbiota), have shown an abundance of AMR genes coding for macrolide, fluroquinolones and tetracycline resistance (Almeida et al., 2020, Carr et al., 2020, Lancaster et al., 2003). Additionally, studies have shown that antibiotic classes used in patients with penicillin allergy are more likely to select out resistance in the oral and gut microbiota. Administration of azithromycin has been linked to increases in resistance genes to both macrolide and non-macrolide (aminoglycosides, beta-lactams, trimethoprim, and metronidazole) antibiotics found in the gut microbiome (Doan et al., 2020). Garcia-Rey et al demonstrated that rates of co-resistance to erythromycin and penicillin in *Streptococcus pneumoniae* was significantly more associated with the use of macrolides compared to β-lactam antibiotics (such as penicillins and cephalosporins) (Garcia-Rey et al., 2002). Additionally, the use of cephalosporins is more likely to select for penicillin resistance in *Streptococcus pneumoniae* when compared to aminopenicillins (Granizo et al., 2000, Garcia-Rey et al., 2002). An experimental mouse model found that treatment for urinary tract infection with ciprofloxacin or fosfomycin was more likely to select for resistance genes to multiple classes of antibiotics in the gut microbiome when compared with ampicillin (Xu et al., 2020).

Justification for study:-

- Poor antimicrobial stewardship is prevalent in treating patients with COVID-19
- Penicillin allergy leads to increased antibiotic use, treatment failure and AMR.
- Antibiotics used in place of narrow spectrum penicillin antibiotics in patients with penicillin allergy have a greater tendency to select for resistant bacteria.
- Higher prevalence of AMR in the oral and GI tract microbiota may be contributing to higher rates of treatment failure and death seen in patients with penicillin allergy.
- Understanding the resistome of patients with penicillin allergy will improve antibiotic prescribing and antibiotic stewardship.

We plan to further investigate the relationships between antimicrobial resistance, penicillin allergy and patient outcomes in a programme of work that will run in conjunction with the ALABAMA trial. Our hypothesis is that patients with a penicillin allergy record are more likely to have resistance to non-penicillin antibiotics in their normal flora and that this will increase their risk of treatment failure with non-penicillin antibiotics. In particular, penicillin allergic patients admitted with COVID-19 who have bacterial infections will have an increased risk of treatment failure and poor outcomes.

Shotgun metagenomics is the untargeted sequencing of all microbial genomes present in a sample. This method allows the detection of all micro-organisms present (including uncultivable bacteria) as well as enabling detection of AMR genes. We will utilise metagenomic methods including meta-transcriptomics which will enable analysis of differential gene expression to give use an in-depth profile of the microbiota as well as an inference of metabolic activity through gene expression. We will also undertake phenotypic validation of the inferred genotypic results using traditional culture and antibiotic susceptibility testing methods.

2. AIM & OBJECTIVES

Aim

IMPAART will investigate the impact of penicillin allergy on AMR and health outcomes in patients with COVID-19.

Objectives

- To determine if, in patients admitted with COVID-19, the presence of a penicillin allergy record impacted on antibiotic prescribing, the rate of treatment failure of bacterial infections, length of hospital stay, *Clostridioides difficile infection* and mortality.
- To profile the resistome in patients with a penicillin allergy record
- To describe the differences in the presence and diversity of resistance genes in the oral and gastrointestinal microbiota of patients with and without a penicillin allergy record.
- To determine if the abundance of antibiotic resistance genes can be reversed/reduced by correcting incorrect penicillin allergy records.

Feasibility objectives

- To determine how many ALABAMA trial participants are willing to consent to giving saliva/stool samples
- To determine how many ALABAMA trial participants who have consented to saliva/stool sample collection actually provide saliva samples (during both face to face consultation and/or via the post)
- To determine how many saliva/stool samples that are collected can be analysed by metagenomics and transcriptomics

- To determine the practicality of obtaining samples within specific time frames.

3. STUDY DESIGN

This research will be conducted in three work streams (WS); WS1 will use data collected during the NIHR funded PEACH study and WS2 and 3 will run alongside the ALABAMA trial 'AntiBiotics, Allergy and Microbial ResistAnce':

WS 1 - A retrospective cohort study to determine how penicillin allergy impacts on antibiotic use and clinical outcomes in patients admitted with COVID-19 (Objective 1). This work stream is named IMPAART-C: A study of the Impact of Penicillin Allergy on Antimicrobial Resistance and health outcomes in patients with COVID-19.

WS 2 - A cross-sectional study to investigate differences in the carriage of antimicrobial resistance genes in the oral and GI flora of patients with and without a penicillin allergy record (Objectives 2,3 5-9). This study is a separate but related study to the ALABAMA trial.

WS 3 - A feasibility study to investigate whether AMR genes are lost in patients who have their penicillin allergy label removed (Objectives 4, 5-9.) This study is related to the ALABAMA trial and will run in conjunction with it.

3.1 WS1 outcomes

Primary outcome

- Mortality rates (day 60) between patients with and without PenA

Secondary outcomes

- Length of hospital stay
- ICU admission rates
- Total antibiotic usage (including antibiotic agent, route of administration and durations)
- Treatment failure (defined as re-prescription of an antibiotic within 28 days of index antibiotic prescription)
- Number of patients with *Clostridioides difficile* infection
- Mortality rates (day 30) between patients with and without PenA
- Number of patients with infections with AMR organisms (Presence antimicrobial resistant secondary bacterial infection)

3.2 WS2 outcomes

Primary outcome

- The prevalence of antibiotic resistance genes in the mouth and GI tract of patients with and without a penicillin allergy record.

Secondary outcomes

- The measure the difference in diversity of antimicrobial resistance genes in the mouth and GI tract of patients with and without a penicillin allergy record.
- The number of resistance genes to specific antibiotic classes present in the mouth and GI tract of patients with and without a penicillin allergy record.
- To determine whether a metagenomic or transcriptomic approach could be used to predict phenotypic antibiotic susceptibility

3.3 WS3 outcomes

Feasibility outcomes

- The number of patients recruited to ALABAMA who consent to participate in the sub-study
- The number of participants who provide a sample at baseline
- The number of participants who provide a second sample > 6 months after enrolment
- The relationship between ARG abundance and treatment response failure
- The relationship between ARG richness and treatment response failure.

Clinical outcomes:

The primary outcome is the change in antibiotic resistance gene abundance over time between trial arms, in the subset of trial patients who have at least one antibiotic prescription prior to their second sampling

Secondary outcomes

- To measure the change in the abundance and richness of antimicrobial resistance genes over time between trial arms
- The relationship between ARG abundance & richness, and treatment response failure (defined as re-presentation with worsening or non-resolving symptoms following treatment with an antibiotic up to 28 days after initial antibiotic prescription)
- The number and abundance of ‘non-penicillin’ antibiotic resistance genes
- The number and abundance of ‘penicillin’ resistance genes

4. STUDY PARTICIPANTS

4.1 WS1 participants

PEACH study (HTA Project: NIHR132254 - Procalcitonin: Evaluation of Antibiotic use in COVID-19 Hospitalised patients (PEACH); https://fundingawards.nihr.ac.uk/award/NIHR132254) participants, which includes adults and adolescents 16 years old and older, admitted to hospital between 1/2/20 and 30/06/20 with a positive SARS-CoV-2 test.

Patients with their allergy status missing will be excluded, and the numbers will be recorded and presented.

4.2 WS2 participants

Inclusion (enrolled participants must fulfil all of the inclusion criteria)

- Adult (≥18 years) patients with a penA or a matched* patients without a penA
- Received antibiotics in the previous 24 months, but not currently receiving or recently treated with (within 28 days) antibiotics
- Willing to provide saliva +/- stool samples

* non-penA patients will be matched by age (within 5 years) and sex.

Exclusion

- Patients unable to give informed consent or who are unwilling/unable to provide saliva samples

Target population: 35 participants PenA and 35 non-PenA

4.3 WS3 participants

Inclusion

- Patients enrolled into the ALABAMA trial who are willing to provide samples for the sub-study

Exclusion

- Patients unable to give informed consent or who are unwilling/unable to provide saliva samples

5. SCREENING AND RECRUITMENT

5.1 WS2 screening and recruitment

Participants will be recruited from patients attending the clinical immunology and allergy outpatient clinics and pre-operative assessment clinics at Leeds Teaching Hospitals NHS Trust.

Clinic lists will be screened and if a patient is eligible they will be invited to participate during their clinic visit by a member of their direct care team who will refer them to a member of the study team who will be available during the visit. The participant will then be given a patient information sheet (PIS) and the study will be discussed with them. Informed consent will be taken after the patient has had time to consider the PIS and ask any questions. Consent will be taken by a member of the IMPAART research team. Consent must be taken prior to the collection of any study samples.

5.2 WS3 screening and recruitment

All participants recruited for the ALABAMA study will receive relevant information regarding this sub-study during the ALABAMA trial baseline call or their PAAP clinic appointment (see appendix 2). If they express interest in taking part in the sub-study, they will be able to give full informed consent (written or verbal) after discussing the study in full detail with the IMPAART or ALABAMA research nurse or doctor.

6. STUDY PROCEDURES

6.1 Sample collection for WS 2 and 3

Baseline samples will be collected up to 6 weeks after enrolment. Participants in WS3 will be required to provide a second sample > 6 months after enrolment or PAAP test.

Samples required: -

- 2x Saliva in OMNIgene ORAL™ Saliva collection tubes for metagenomic and transcriptomics
- 1x Saliva in Universal container for culture and antibiotic susceptibility testing (WS 2 only)
- 1x Stool sample using a gFOBT (guaiac Fecal Occult Blood test) card.

The saliva sample tubes and gFOBT cards will be provided together with sample collection instructions and will either be given directly to participants when recruited or posted out to them.

Once participants collect their samples, they will post back the specimen using pre-paid postage boxes.

Participants who are collecting samples at home, will receive a text messages reminder if samples are not returned within 2 to 3 weeks after receipt of the testing kit. Another text message reminder will be sent 2 to 3 weeks after the initial reminder text. If required three attempts will be made to contact participant by telephone if sample has not been sent in 28 days from initial text message. A participant will be considered to be “lost to follow up” if they do not provide a sample after these reminders.

Samples will be stored in the University of Leeds Dental Institute. Samples will be processed both at the Dental Institute. and the Leeds Institute of Medical Research at the University of Leeds.

6.2 Data collection

Patient electronic medical records (PPM+ and SystmOne) will be accessed to obtain clinical data including age, sex, prior antibiotic use, co-morbidities and penicillin allergy history. Patient records (via System One) will be accessed to obtain clinical data including age, sex, prior antibiotic use, co-morbidities and antibiotic treatment.

Where applicable treatment outcomes (success and failure) will be obtained from ALABAMA trial data.

6.3 Culture and traditional (phenotypic) antibiotic susceptibility testing (saliva samples only)

Upon receipt samples will be refrigerated and then processed within 72hrs. Saliva samples will be inoculated on to selective streptococci and staphylococci agar and incubated. Susceptibility testing will be done using disk diffusion methods as per the European Committee on Antimicrobial susceptibility testing (EUCAST).

6.4 Metagenomic sample handling and processing

Samples will be stored at -80 degrees Celcius in the UoL and batch processed. DNA will be extracted using ZymoBIOMICS DNA Mini Kit and DNA sequenced the Illumina® Nextseq platform.

6.5 Transcriptomic sample handling and processing (saliva samples only)

Sample processing and analysis will occur in UoL laboratories. Samples will stored at −80°C as per manufacturer instructions until further processing. RNA will be extracted using ZymoBIOMICS™ RNA Mini Kit as per manufacturer protocol and will then be processed in batch using the NextSeq (Illumina®) platform.

6.6 Participant follow up (WS 3 only)

Participants will be followed up as per the ALABAMA Study protocol. Patients will be contacted by post and by text message to remind them to send required follow up samples. Participants will receive a text messages reminder if samples are not returned within 2 to 3 weeks after receipt of the testing kit. Another text message reminder will be sent 2 to 3 weeks after the initial reminder text. In addition to this, where required three attempts will be made to contact patient by telephone if participants have not provided the follow-up sample 28 days from initial text message. A participant will be considered to be “lost to follow up” if they do not provide a sample after these reminders.

6.7 Schedule of procedures WS 2 and 3

Procedures	Screening	Baseline visit (face to face or via telephone)	Follow up (WS 3 only)
Informed consent		X	
Assessment of eligibility	X	X	
Sample collection		X	
Review of medical and antibiotic history		X	

Second sample collection			X
Review of clinical outcomes			X

6.8 Excess sample storage / Biobanking

Where applicable, any surplus samples will be stored and may be used for future research by researchers from Leeds Teaching Hospitals NHS Trust, the University of Leeds, as well as researchers from other institutions and commercial companies both in and outside the UK for ethically approved studies. This may include transfer of samples to a research tissue bank.

7. STATISTICAL ANALYSIS

7.1 Sample size

7.1.1. Work stream 1: All eligible patients in the PEACH study will be included

7.1.2. Work stream 2: A sample size of 35 patients in each arm is acceptable for pilot studies (Lancaster et al., 2004, Browne, 1995) and although this is not a definitive study, this sample size will provide 80% power to see a standardised effect size of 0.68 for a continuous measure of antimicrobial resistance. This will give a medium to large effect size (Cohen, 1988).

7.1.3. Work stream 3: No formal sample size has been calculated as this is a pilot study, however all patients recruited into the ALABAMA study from sites in Leeds or West Yorkshire are eligible for recruitment.

7.2 Description of statistical methods

Baseline characteristics will be descriptive, with categorical data presented as proportions (%) and continuous data as medians and interquartile ranges (IQR) or as means and standard deviations (SD). Multivariable logistic regression will be used to assess which clinical variables (age, sex, co-morbidity, PenA status and previous antibiotic history) are associated AMR).

7.3 DNA sequence analysis

The high-performance computer (HPC) infrastructure available at the University of Leeds (https://arc.leeds.ac.uk/) will be used to carry out metagenomic analyses of the microbial

nucleotide sequence data (in FASTQ file format). Following quality trimming using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/,) de novo gene assembly will be carried out on the read sequences using diamond (http://ab.inf.uni-tuebingen.de/software/diamond/) and MG-RAST (https://www.mg-rast.org/) servers. Taxonomical and gene annotations will be done using the large NCBI non-redundant protein database (https://www.ncbi.nlm.nih.gov/guide/proteins/). The resistome profile will also be determined by mapping DNA contigs against the ARDB (antibiotic resistance genes database, https://card.mcmaster.ca/ and https://ardb.cbcb.umd.edu/). Significant difference in functional capability between sample groups will tested with the Wald test using the DESeq2 R package (http://bioconductor.org/packages/release/bioc/html/DESeq2.html).

8. ETHICAL PRINCIPLES

These sub-studies will be conducted in line with the principles of the Declaration of Helsinki and conducted in accordance with relevant regulations and with Good Clinical Practice.

The study protocols, patient information sheets and consent forms will be submitted to the Research Ethics Committee (REC) for approval prior to study initiation.

Upon completion of the study, samples may be transferred to a Research Tissue Bank (RTB) licensed by the Human Tissue Authority.

8.1 Informed consent

Written informed consent will be obtained from participants in WS 2 and those in WS 3 who will be seen in the ALABAMA trial clinic for allergy testing. Potential participants will be given a Participant Information Sheet (PIS) and Informed Consent Form (ICF) to read, this will then be followed up with a discuss with a member of the IMPAART or ALABAMA study team who will be suitably qualified and experienced in taking informed consent.

The potential participant will be given opportunities to ask questions and allowed as much time as they need consider the information. Where applicable participants attending ALABAMA trial clinics will have the PIS and consent form sent to them alongside their ALABAMA clinic letter.

A copy of the completed Informed Consent Form (ICF) will be given to the participant and another filed in their medical notes. The original form will be retained in the study site file at the Leeds Teaching Hospitals NHS Trust.

For patients who are recruited into WS 3 who will not have a face to face clinic appointment, verbal consent will be sought. A written Participant Information Sheet (PIS) and Informed Consent Form (ICF) will have been sent by post or email to the participant prior to the telephone consent appointment. During this telephone discussion, a member of the IMPAART study team will go through the PIS, the ICF and discuss the exact nature of the study; what it will involve for the participant; the potential risks and benefits involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care or involvement in the ALABAMA trial, and without affecting their legal rights, and with no obligation to give the reason for withdrawal. The participant will be given the opportunity to ask questions and will be allowed as much time as required to consider the information. If needed a follow up call can be arranged to take consent. If patient is willing to participate during the call, verbal consent will be obtained and recorded in a consent form that will be signed and dated by the IMPAART study team member taking consent.

The original form will be retained in the study site file at the Leeds Teaching Hospitals NHS Trust. A copy of the completed Informed Consent Form (ICF) a copy will be filed in their medical notes and another copy will then be sent to the participant. They will be asked to sign a return slip to confirm their participation. The original ICF form completed via telephone consultation will be retained in the study site file at the Leeds Teaching Hospitals NHS Trust. A copy of this telephone completed ICF will be filed in the participants medical notes and another sent to the participant. Verbal consent may also be sought for any potential participants in WS2 or WS3 who during their clinic visit state that they wish to take more time to consider study participation. For these participants a follow up call will be arranged so that any questions can be answered and verbal consent obtained if the participants are willing.

9. DATA PROTECTION

All participant data will be handled in compliance with the General Data Protection Regulation (GDPR) and Data Protection Act 2018. All study staff will ensure that the participants' anonymity is maintained. All data will be anonymised as soon as it is practical to do so and participants will be identified by a participant ID number on all study documents. All documents will be stored securely on either NHS or University of Leeds computers and will only be accessible by study staff and authorised personnel. All research data will be retained for 5 years beyond the end of the study. If participants give consent any excess samples may be retained and stored in a research tissue bank. Otherwise, samples collected from participant samples will be retained for 12 months after study end for verification and quality checking of research data, after which they will be destroyed in accordance with the Human Tissue Authority's Code of Practice. All personal identifiable information will be removed from stored samples.

10. REFERENCES

ALMEIDA, V. S. M., AZEVEDO, J., LEAL, H. F., QUEIROZ, A. T. L., DA SILVA FILHO, H. P. & REIS, J. N. 2020. Bacterial diversity and prevalence of antibiotic resistance genes in the oral microbiome. *PLoS One,* 15**,** e0239664.

BAXTER, M., BETHUNE, C., POWELL, R. & MORGAN, M. 2020. Point prevalence of penicillin allergy in hospital inpatients. *J Hosp Infect,* 106**,** 65-70.

BLUMENTHAL, K. G., LU, N., ZHANG, Y., LI, Y., WALENSKY, R. P. & CHOI, H. K. 2018. Risk of meticillin resistant Staphylococcus aureus and Clostridium difficile in patients with a documented penicillin allergy: population based matched cohort study. *BMJ,* 361**,** k2400.

BROWNE, R. H. 1995. On the use of a pilot sample for sample size determination. *Stat Med,* 14**,** 1933-40.

CARR, V. R., WITHERDEN, E. A., LEE, S., SHOAIE, S., MULLANY, P., PROCTOR, G. B., GOMEZ-CABRERO, D. & MOYES, D. L. 2020. Abundance and diversity of resistomes differ between healthy human oral cavities and gut. *Nat Commun,* 11**,** 693.

COHEN, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*, Routledge.

DOAN, T., WORDEN, L., HINTERWIRTH, A., ARZIKA, A. M., MALIKI, R., ABDOU, A., ZHONG, L., CHEN, C., COOK, C., LEBAS, E., O'BRIEN, K. S., OLDENBURG, C. E., CHOW, E. D., PORCO, T. C., LIPSITCH, M., KEENAN, J. D. & LIETMAN, T. M. 2020. Macrolide and Nonmacrolide Resistance with Mass Azithromycin Distribution. *N Engl J Med,* 383**,** 1941-1950.

GARCIA-REY, C., AGUILAR, L., BAQUERO, F., CASAL, J. & DAL-RE, R. 2002. Importance of local variations in antibiotic consumption and geographical differences of erythromycin and penicillin resistance in Streptococcus pneumoniae. *J Clin Microbiol,* 40**,** 159-64.

GRANIZO, J. J., AGUILAR, L., CASAL, J., GARCIA-REY, C., DAL-RE, R. & BAQUERO, F. 2000. Streptococcus pneumoniae resistance to erythromycin and penicillin in relation to macrolide and beta-lactam consumption in Spain (1979-1997). *J Antimicrob Chemother,* 46**,** 767-73.

LANCASTER, G. A., DODD, S. & WILLIAMSON, P. R. 2004. Design and analysis of pilot studies: recommendations for good practice. *J Eval Clin Pract,* 10**,** 307-12.

LANCASTER, H., READY, D., MULLANY, P., SPRATT, D., BEDI, R. & WILSON, M. 2003. Prevalence and identification of tetracycline-resistant oral bacteria in children not receiving antibiotic therapy. *FEMS Microbiol Lett,* 228**,** 99-104.

LANGFORD, B. J., SO, M., RAYBARDHAN, S., LEUNG, V., SOUCY, J. R., WESTWOOD, D., DANEMAN, N. & MACFADDEN, D. R. 2021. Antibiotic prescribing in patients with COVID-19: rapid review and meta-analysis. *Clin Microbiol Infect*.

LANGFORD, B. J., SO, M., RAYBARDHAN, S., LEUNG, V., WESTWOOD, D., MACFADDEN, D. R., SOUCY, J. R. & DANEMAN, N. 2020. Bacterial co-infection and secondary infection in

patients with COVID-19: a living rapid review and meta-analysis. *Clin Microbiol Infect,* 26**,** 1622-1629.

LANSBURY, L., LIM, B., BASKARAN, V. & LIM, W. S. 2020. Co-infections in people with COVID-19: a systematic review and meta-analysis. *J Infect,* 81**,** 266-275.

MACY, E. & CONTRERAS, R. 2014. Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: A cohort study. *J Allergy Clin Immunol,* 133**,** 790-6.

RAWSON, T. M., MOORE, L. S. P., ZHU, N., RANGANATHAN, N., SKOLIMOWSKA, K., GILCHRIST, M., SATTA, G., COOKE, G. & HOLMES, A. 2020. Bacterial and Fungal Coinfection in Individuals With Coronavirus: A Rapid Review To Support COVID-19 Antimicrobial Prescribing. *Clin Infect Dis,* 71**,** 2459-2468.

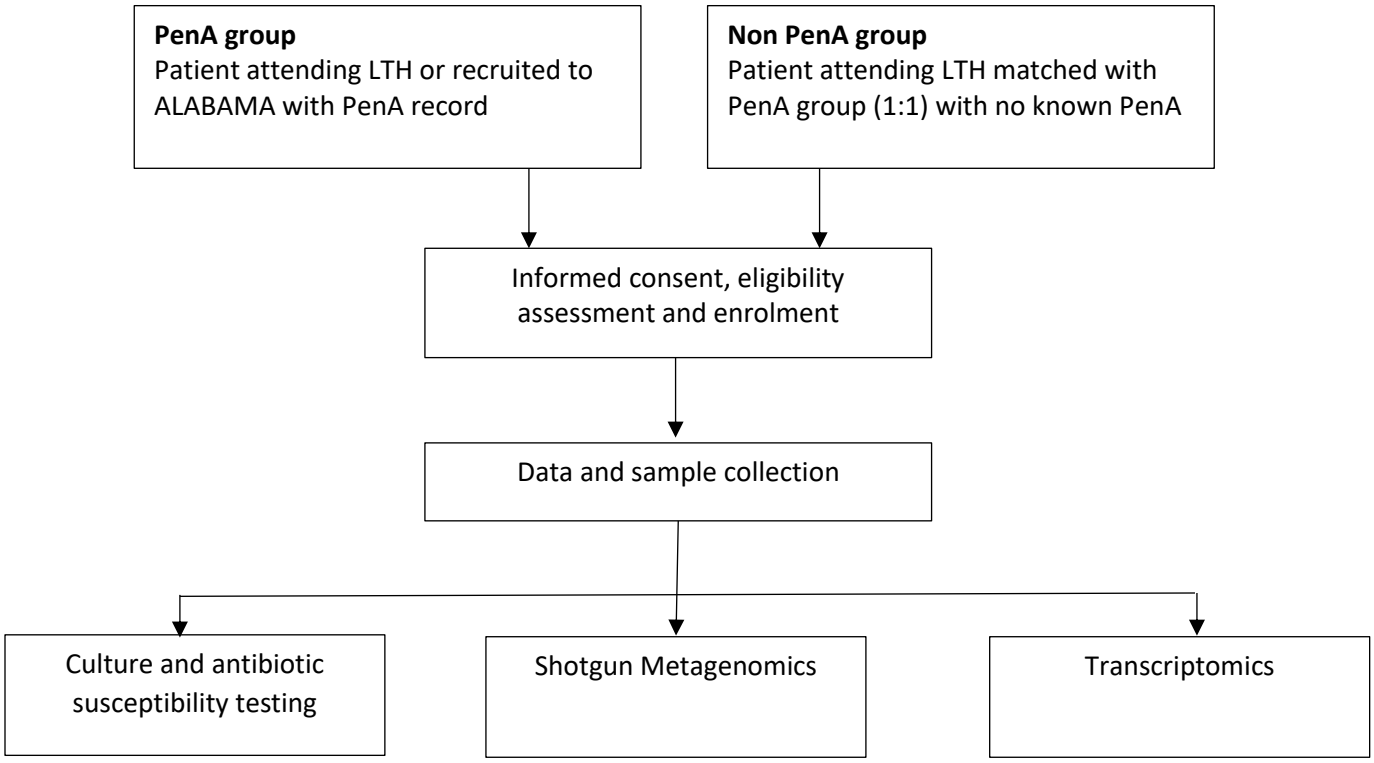
SHENOY, E. S., MACY, E., ROWE, T. & BLUMENTHAL, K. G. 2019. Evaluation and Management of Penicillin Allergy: A Review. *JAMA,* 321**,** 188-199.

WEST, R. M., SMITH, C. J., PAVITT, S. H., BUTLER, C. C., HOWARD, P., BATES, C., SAVIC, S., WRIGHT, J. M., HEWISON, J. & SANDOE, J. A. T. 2019. 'Warning: allergic to penicillin': association between penicillin allergy status in 2.3 million NHS general practice electronic health records, antibiotic prescribing and health outcomes. *J Antimicrob Chemother,* 74**,** 2075-2082.

XU, L., SURATHU, A., RAPLEE, I., CHOCKALINGAM, A., STEWART, S., WALKER, L., SACKS, L., PATEL, V., LI, Z. & ROUSE, R. 2020. The effect of antibiotics on the gut microbiome: a metagenomics analysis of microbial shift and gut antibiotic resistance in antibiotic treated mice. *BMC Genomics,* 21**,** 263.

11. APPENDIX

Appendix 1: Workstream 2 patient flow



Appendix 2: Workstream 3 recruitment pathway (**ALABAMA Trial procedures represented in bold)**