Genotypic detection of antimicrobial resistance in *S. pneumoniae* isolates using multiple PCR methods

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01 Introduction & background

This research project is part of the **LAKANA trial**, a mass-drug administration (MDA) of azithromycin, an antibiotic, to 1- to 11-month-old children in Mali, West Africa, aimed at reducing the high child mortality rate.

Despite the benefit of promoting survival, the MDA of antibiotics poses the risk of **antimicrobial resistance (AMR)** developing in bacteria. This is a major global health concern, as antibiotics could eventually lose their effectiveness against certain bacteria, putting a person's life at risk in the case of a serious bacterial infection. Hence, monitoring the prevalence of AMR as the trial proceeds is necessary. We need to ensure that this potential issue does not outweigh the advantages of MDA.

Project overview

- We genotypically tested 24 *S. pneumoniae isolates* for resistance against **azithromycin** and **penicillin** by performing **qPCR** and colorimetric **LAMP assay**. The isolates were obtained from the nasopharyngeal swabs of the trial participants in Mali and have been phenotypically tested for resistance against multiple antibiotics.
- Genotype = the genetic makeup of an organism; phenotype = the physical characteristics manifested from the genotype.
- Phenotypic testing shows the nature of bacteria in the presence of antibiotics, whereas genotypic testing enables us to see the specific genes and modes of action related to AMR.
- We **compared the obtained genotypic data with the respective phenotypic data** to see if any discrepancies occur.
- We also estimated and **compared the sensitivity of qPCR and LAMP assay** using **ddPCR** as a reference.
- We are interested in **seeing the accuracy of LAMP (loop-mediated isothermal amplification) assay**, as this method, if proven successful, would be more suitable for use in clinical trials in resource-limited settings, including the LAKANA trial in Mali. This is because it is quick (<30 minutes), easy to read out (result observable by the eye), relatively cheap, and requires less equipment and expertise compared to other PCR methods.

02 Methods

Bacterial culturing → DNA extraction & quantification → Automated electrophoresis (TapeStation) → Quantitative PCR (qPCR)

Automated electrophoresis (TapeStation) → Droplet Digital PCR (ddPCR)

Automated electrophoresis (TapeStation) → Colorimetric Loop-mediated isothermal amplification (LAMP)

Quantitative PCR (qPCR)

1. Made standard curves with dilution series of lab strains (by a factor of 10) to determine limit of detection
2. Tested extracted samples

Droplet Digital PCR (ddPCR)

Performed on dilution series of lab strains

Colorimetric Loop-mediated isothermal amplification (LAMP)

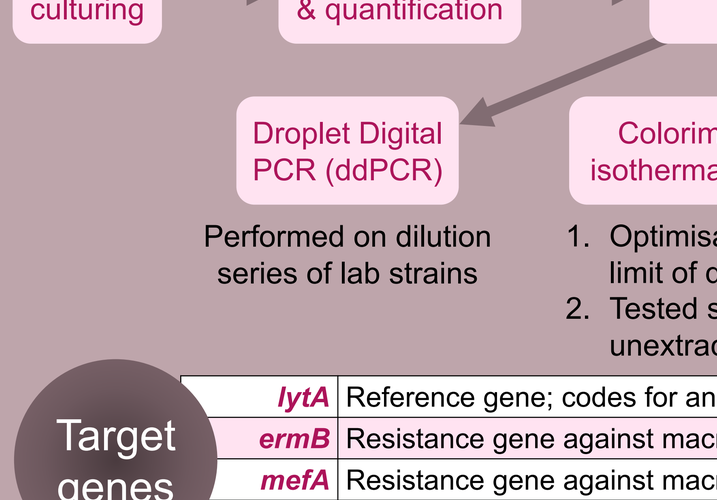
1. Optimisation and determining limit of detection
2. Tested samples (extracted and unextracted)

Target genes

lytA	Reference gene; codes for an autolysin specific to *S. pneumoniae*
ermB	Resistance gene against macrolide; codes for methylase enzyme
mefA	Resistance gene against macrolide; codes for active efflux pumps
pbp2b	Susceptibility gene to β-lactam; codes for penicillin-binding protein

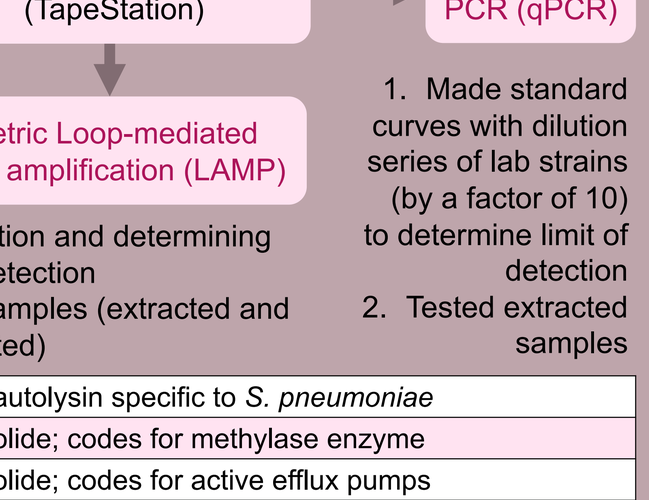
03 Results

qPCR



The amplification graphs for all 24 samples with a 10^{-2} dilution over 45 cycles. Four genes were targeted: *lytA* (blue), *pbp2b* (red), *ermB* (purple), and *mefA* (green). The fluorescence of the *lytA*, *pbp2b*, *ermB*, and *mefA* gene probes are detected in the FAM, Texas Red, Cy5, and HEX channels, respectively.

LAMP assay (extracted samples)



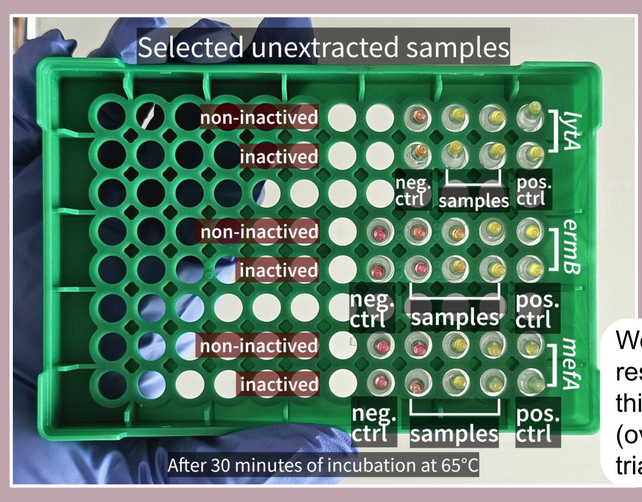
The LAMP assay result for all 24 extracts with a 10^{-2} dilution. Yellow indicates a positive result, whereas pink indicates a negative result. Three genes were targeted: *lytA* (rows A & B), *ermB* (rows C & D), and *mefA* (rows E & F).

qPCR and LAMP assay results comparison

***lytA* gene**	qPCR positive	qPCR negative	***ermB* gene**	qPCR positive	qPCR negative	***mefA* gene**	qPCR positive	qPCR negative
LAMP positive	24	0	LAMP positive	2	0	LAMP positive	5	0
LAMP negative	0	0	LAMP negative	1	21	LAMP negative	0	19

Comparison of the qPCR and LAMP assay results for the same sample dilution of 10^{-2}. Overall, for the tested 24 samples, the two methods show great consistency in the results. Only one sample was observed to be qPCR-positive but LAMP-negative for the *ermB* gene.

LAMP assay (selected unextracted samples)



The LAMP assay result for selected unextracted samples. For each primer set, two methods were tested: non-inactivated and inactivated (in PBS and heat). From the picture, it is evident that the two methods give very similar outcomes. It is also observed that the actual results align with the expected results, showing that the LAMP assay worked well for unextracted samples in this case.

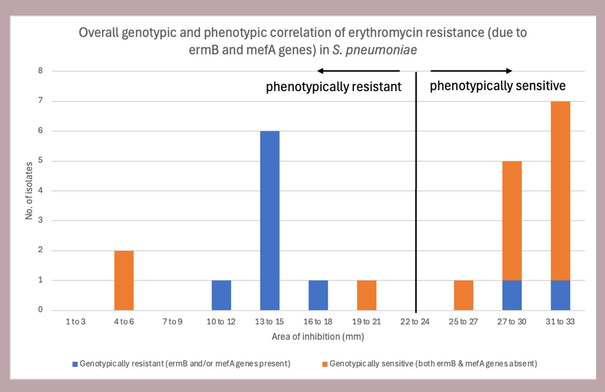
We are interested in seeing the LAMP result for unextracted samples since this approach would be preferrable (over extracted samples) in clinical trials due to its convenience.

ddPCR (sensitivity determination)

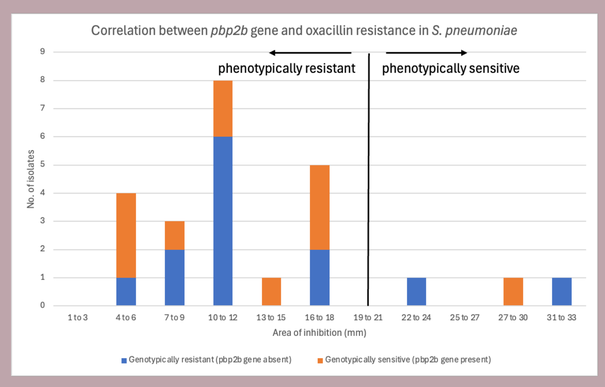
PCR method	Estimated sensitivity (DNA copies/µl)
qPCR	6
LAMP assay	50

Comparison of qPCR and LAMP assay sensitivity. qPCR is revealed to be about 8x more sensitive than LAMP assay.

Genotypic-phenotypic comparison



Genotypic and phenotypic correlation for erythromycin resistance. The graph reveals that genotypic-phenotypic discrepancies occurred. 3 out of 11 (27.3%) phenotypically resistant isolates are genotypically sensitive, and 2 out of 13 (15.4%) phenotypically sensitive isolates are genotypically resistant. If the genotypic and phenotypic data fully match, we would expect the bars to the left of the borderline to all be blue, whereas those to the right of the borderline would all be orange.



Genotypic and phenotypic correlation for oxacillin resistance. The graph reveals that genotypic-phenotypic discrepancies occurred. 10 out of 21 (47.6%) phenotypically resistant isolates are genotypically sensitive, and 2 out of 3 (66.7%) phenotypically sensitive isolates are genotypically resistant. If the genotypic and phenotypic data fully match, we would expect the bars to the left of the borderline to all be blue, whereas those to the right of the borderline would all be orange.

04 Discussion

- Through the multiple PCR experiments, we successfully obtained the genotypes of 24 *S. pneumoniae* isolates and compared them with the respective phenotypes.
- The PCR experiments can be improved by performing them in duplicates or triplicates.
- Further steps that can be done include DNA sequencing, MALDI-TOF mass spectrometry and whole genome sequencing.
- Most importantly, we need to test the remaining samples to establish a more accurate genotype and phenotype comparison.
- To determine the suitability of LAMP assay for implementation in clinical settings, further tests on sensitivity and specificity are required.

04 Conclusion

- More samples need to be tested for us to be more confident with the results.
- LAMP assay remains an interesting technique to be researched as it has a great potential to be implemented in clinical trials.

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