Monitoring Antimicrobial Resistance in Children Receiving Azithromycin

How can we use LAMP technology to measure AMR in resource-limited settings?

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1. Introduction

This research project is part of the LAKANA trial, which aims to reduce the high child mortality rate in Mali using mass drug administration (MDA) of the macrolide antibiotic azithromycin. [1]

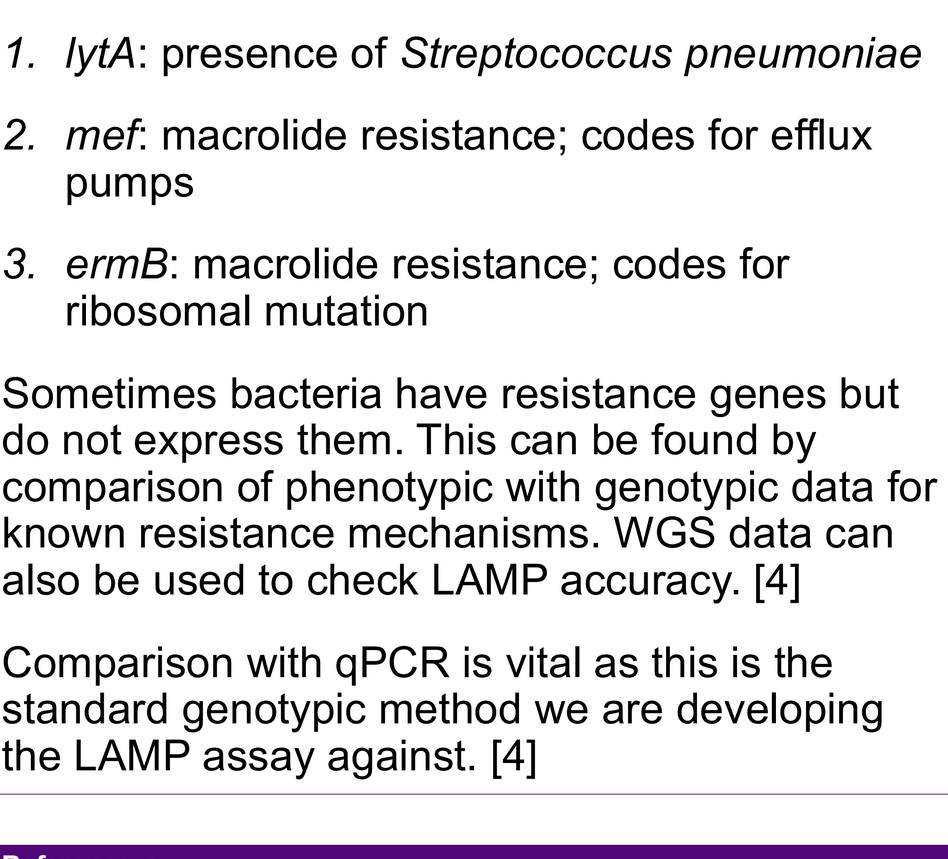
Azithromycin MDA can reduce all-cause child mortality, but also increases antimicrobial resistance (AMR), which causes antibiotics to lose their effectiveness. This is a major global health concern. [1]

Traditional AMR detection methods (e.g. qPCR, phenotypic methods) are difficult in resource-limited settings such as Malian villages. LAMP is a simpler, cheaper, and faster method more suited to these non-laboratory settings. [1]

Research questions

1. How can we optimise the conditions of the LAMP assay?
2. How does the LAMP assay compare to other AMR detection methods?

2. Method



Target genes: [2,3]

1. *lytA*: presence of *Streptococcus pneumoniae*
2. *mef*: macrolide resistance; codes for efflux pumps
3. *ermB*: macrolide resistance; codes for ribosomal mutation

Sometimes bacteria have resistance genes but do not express them. This can be found by comparison of phenotypic with genotypic data for known resistance mechanisms. WGS data can also be used to check LAMP accuracy. [4]

Comparison with qPCR is vital as this is the standard genotypic method we are developing the LAMP assay against. [4]

3. Results

LAMP assay result

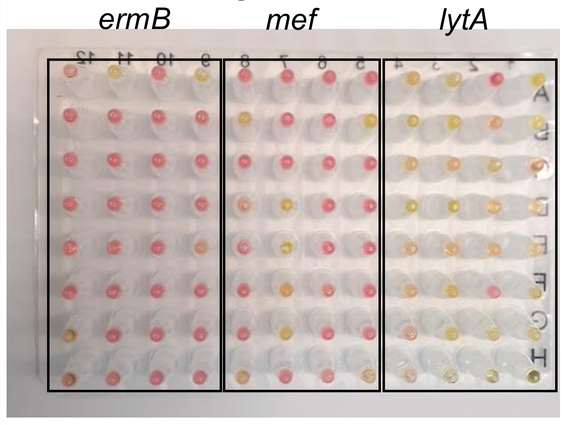


Figure 1 LAMP assay testing plate
Pink is positive, yellow is negative

qPCR result

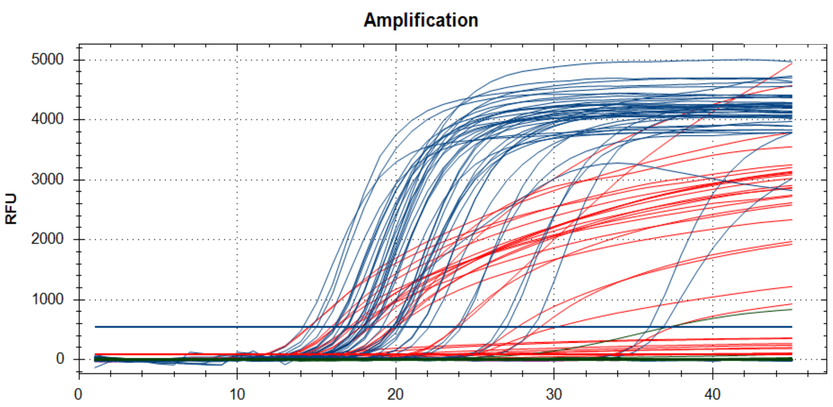


Figure 2 qPCR duplex result. Curve is positive, flat line is negative

LAMP versus qPCR

		LAMP					
		lytA		*mef*		*ermB*	
		+ve	-ve	+ve	-ve	+ve	-ve
qPCR	+ve	91	5	3	4	5	1
	-ve	13	3	9	94	3	97

Figure 3 Collated comparison of qPCR and LAMP results for *lytA*, *mef*, and *ermB*

LAMP versus Phenotypic Testing

		LAMP	
		+ve	-ve
Pheno	+ve	7	9
	-ve	16	80

Figure 4 Comparison of resistance found in phenotypic and LAMP.

Temperature

Target gene	65°C	63°C	61°C
lytA	✓	X	✓
mefA	✓	X	✓
ermB	X	✓	✓

Figure 5 Performance of different primer sets at different temperatures.
✓ = no/few intermediate (orange) results
X = many intermediate (orange) results

4. Discussion

- 61°C allows all 3 primer sets to work concurrently, further testing is needed to check consistency of results
- Overall, our LAMP assay works well versus qPCR
  - Issues with *lytA* primers due to qPCR problems
- LAMP also works as expected against phenotypic testing
  - Higher numbers of false positives and negatives expected as genotypic methods usually have lower sensitivity and specificity versus phenotypic methods

5. Further Research

1. Check consistency of results with 61°C condition
2. Re-test qPCR samples to fix *lytA* primer issues
3. Test unextracted samples using nasopharyngeal swabs
4. Test for other bacteria common in the nasopharynx to check for lack of cross reaction (i.e. false positives)
5. Compare intermediate results to phenotypic methods and qPCR, are they positive

References:
[1] Adubra, L., Alber, D., Ashorn, P. *et al* (2023) Testing the effects of mass drug administration of azithromycin on mortality and other outcomes among 1–11-month-old infants in Mali (LAKANA). *Trials* **24**:5
[2] Zahari, N.I.N., Engku Abd Rahman, E.N.S., Irekeola, A.A., Ahmed, N., Rabaan, A.A., Alotaibi, J. et al (2023) A Review of the Resistance Mechanisms for β-Lactams, Macrolides, and Fluoroquinolones among Streptococcus pneumoniae. Medicina (Kaunas) **59**:11
[3] Hajia, M., Farzanehkhah, M., Hajiashemi, B., Dolaytar, A., Imani, M., Saburian, R. *et al* (2014) Real-Time Assay as A Tool for Detecting *lytA* Gene in Streptococcus pneumoniae Isolates *Cell Journal* **16**:2
[4] Zankari, E., Hasman, H., Kaas, R.S., Seyfarth, A.M., Agersø, Y., Lund, O. *et al* (2012) Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. *Journal of Antimicrobial Chemotherapy* **68**:4