Rapid molecular diagnostics for extensively drug-resistant (XDR) *Salmonella* Typhi

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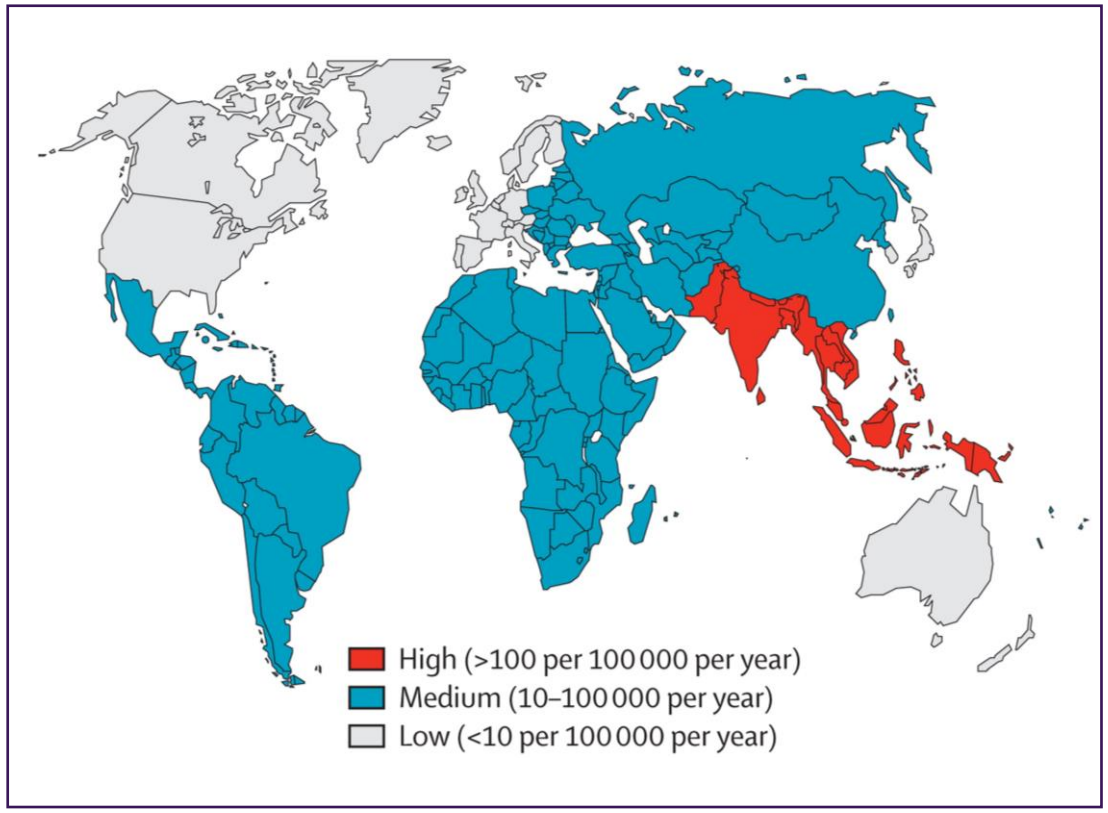
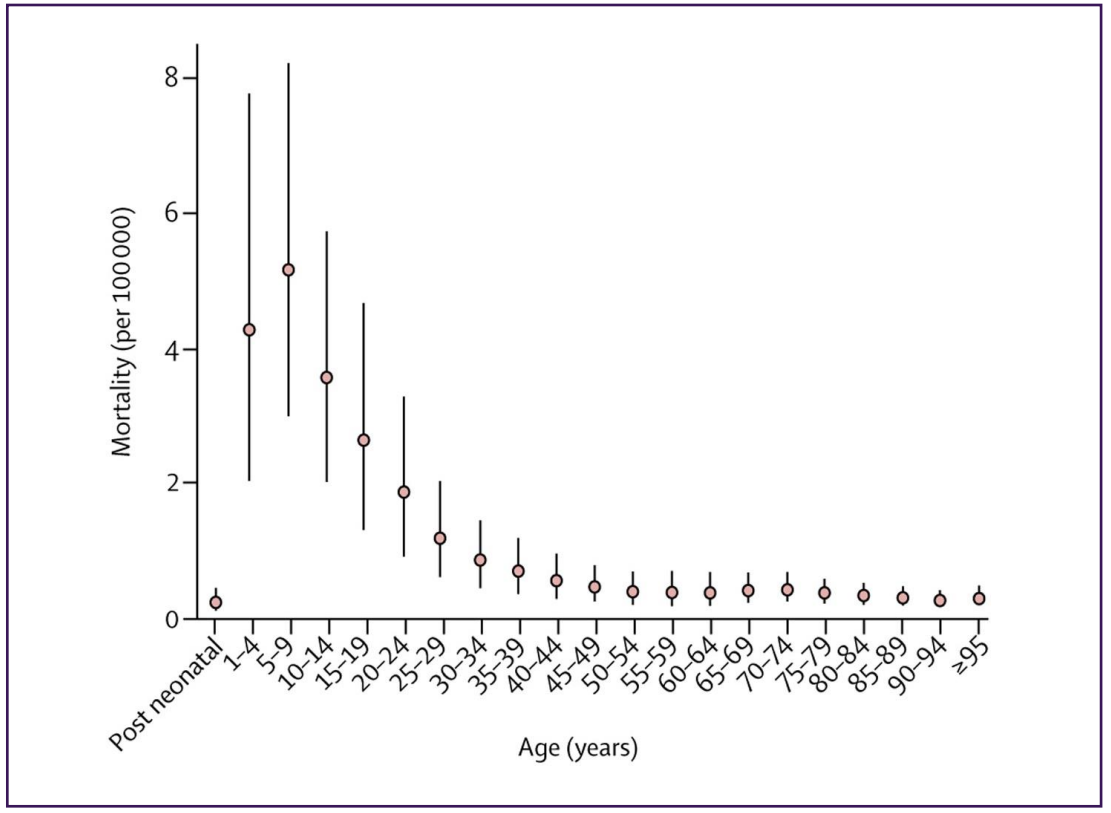
Typhoid fever: a global burden

- Widespread dissemination disproportionately affecting children, predominantly in developing countries.
- Up to 30% mortality rate, with recent emergence of extensively-drug resistant (XDR) strains, resistant to almost all antimicrobials.



Geographical distribution of Typhoid fever.
Source: Maurice, J. (2012). *A first step in bringing typhoid fever out of the closet*. The Lancet, 379:9817, pp 699-700.



Global age-specific mortality rates (per 100 000) from typhoid and paratyphoid fevers in 2017.
Source: GBD 2017 Typhoid and Paratyphoid Collaborators. (2019).*The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017.* The Lancet Infectious Diseases, 19:369–81.

Increased transmission of XDR *S.*Typhi

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RESEARCH ARTICLE

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Emergence of an Extensively Drug-Resistant *Salmonella enterica* Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation Cephalosporins

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Source: Klemm et al. (2018) *Emergence of an Extensively Drug-Resistant Salmonella enterica Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third Generation Cephalosporins. mBio 9:e00105-18. https://doi.org/10.1128/mBio.00105-18.*

A need for improved *S.*Typhi diagnostics

Current diagnostic challenges

- Lack of sensitivity and specificity, particularly at the point-of-care[1].
- Conventional blood cultures relatively expensive and can take > 48 hours.
- Invasive infections (bacteraemia) often have low bacterial burden (~1 CFU/ml of blood)[2]
- Recent emergence of extensive antimicrobial resistance (fluroquinolones and third-gen cephalosporins) means testing unable to identify those at risk of treatment failure.

Improving diagnostic options

- Ideally culture-free with detection directly from patient sample.
- Can be deployed at centralised pathology services right through to point-of-care in low income settings.
- Rapidly (less than a few hours) identify Typhi and guide antimicrobial treatment[1].
- Provide resistance-guided therapy to improve both patient outcomes and antimicrobial stewardship.
- Ultimately, new methods must contribute to reduced morbidity and mortality.

1. Mather, R.G., et al. (2019) *Redefining typhoid diagnosis: what would an improved test need to look like?* BMJ Global Health 4(5): e001831.
2. Wain, J., et al. (2004) *Quantitation of Bacteria in Blood of Typhoid Fever Patients and Relationship between Counts and Clinical Features, Transmissibility, and Antibiotic Resistance* Journal of Clinical Microbiology 36(6): pp 1683-1687.

Improving XDR *S.*Typhi diagnostics

Hypothesis: Molecular assay development will facilitate the rapid detection (in hours) of extensively drug resistant *Salmonella* Typhi and antimicrobial resistance markers, enabling faster patient-centred treatment of *Salmonella* Typhi infections

Aim 1: Design novel molecular detection assays (NAAT) for XDR *S*. Typhi diagnosis

- Use established (real-time PCR) and emerging (isothermal amplification) detection methods based on DNA amplification and detection.
- Validation of novel molecular assays to determine sensitivity and specificity for XDR *S*. Typhi detection.

Aim 2: Perform whole genome sequencing of XDR *S*. Typhi isolates

- Identify any variation or acquisition of AMR determinants.
- Align phenotypic and genotypic data.
- Contribute to the building of a reference genome catalogue of emerging XDR strains.

Novel molecular diagnostics for XDR *S.* Typhi

- We developed three NAA assays for the detection of extensively drug-resistant *Salmonella* Typhi

1. Real-time PCR, ABI7500 (ThermoFisher)

Singleplex assays

Target	Gene/s	Sensitivity	Specificity
Typhi	STY4669	100%	100%
Typhi	*fliC*	100%	100%
Typhi H58	STY1507-08	100%	100%
AMR	blaCTX-M-15	90%	100%
AMR	qnrS1	100%	98%

Multiplex (triplex) assay

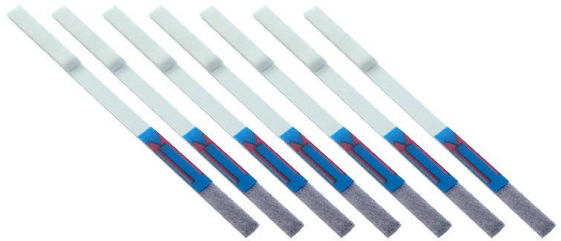
Target	Gene/s	Sensitivity	Specificity
Typhi	STY4669	100%	100%
AMR	blaCTX-M-15	89%	100%
AMR	qnrS1	88%	100%

2. LAMP, Genie III (OptiGene)

Singleplex assays

Target	Gene/s	Sensitivity	Specificity
Typhi	*fliC*	100%	100%
AMR	blaCTX-M-15	94%	91%
AMR	qnrS1	93%	96%

LAMP time-to-result in as little as 8.5 mins



3. RPA-Lateral flow, HybriDetect (Milenia Biotec)

Singleplex assays

Target	Gene/s	Sensitivity	Specificity
Typhi	*fliC*	100%	100%
AMR	blaCTX-M-15	88%	100%
AMR	qnrS1	94%	100%

RPA time-to-result in around 15 mins

Whole genome sequencing of XDR *S.* Typhi

- Inconsistent AMR determinant chromosomal integration and plasmid acquisition.

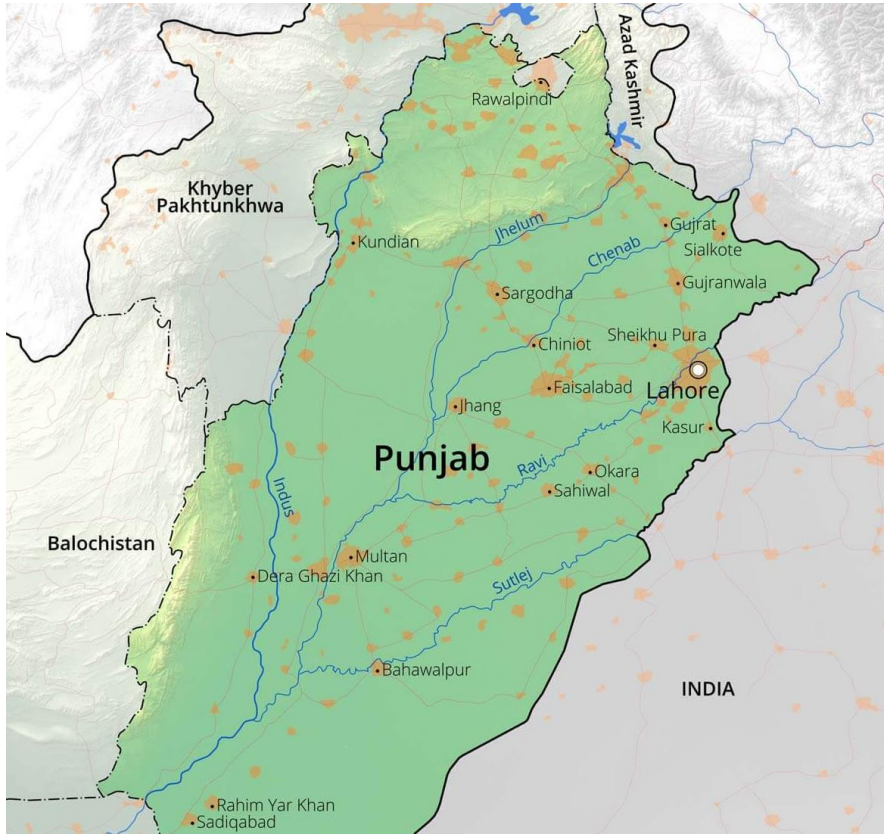
AMR determinant	XDR *Salmonella* Typhi isolate																							
	1		2		3		4		5		6		7		9		11		12		13		16	
	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P
aph(3")-Ib	+	+	+	+		+		+		+	+			+		+	+	+		+	+	+	+	
aph(6)-Id	+	+	+	+		+		+		+	+			+		+	+	+		+	+	+	+	
bla TEM-1B	+	+		+		+		+		+	+		+	+		+	+	+		+	+	+	+	
catA1	+		+		+		+				+		+		+		+		+		+		+	
dfrA7	+		+		+		+				+		+		+		+		+		+		+	
gyrA (S83F)	+		+		+		+		+		+		+		+		+		+		+		+	
sul1	+		+		+		+				+		+		+		+		+		+		+	
sul2	+	+	+	+		+		+		+	+			+		+	+	+		+	+	+	+	
bla CTX-M-15		+		+		+		+	++	+			+	+		+		+		+		+		
qnrS1		+		+		+		+		+				+		+		+		+		+		

A chance to evaluate real-time PCR assays

- **Patient:** child presents at QCH after recently returning from Pakistan, presenting with symptoms of enteric fever
- **Diagnosis**: most-likely invasive infection following gastroenteritis; given travel history significant chance of invasive Salmonellosis (typhoid fever)
- **Treatment**: Ceftriaxone, then Azithromycin
- **Pathology testing**: 4-hour blood culture NEG; stool sample culture NEG, stool NAE Salmonella sp. POS at MDU.
- **UQCCR testing:** NAE of stool sample sent to us for testing (research only) with prototype real-time PCR assay. Results = Typhi target 1 POS, Typhi target 2 POS, Typhi H58 target POS, *bla*$_{\text{CTX-M}}$ group 1 POS, *qnrS* POS.

New XDR *S.* Typhi diagnostics: moving forward

- Further screening of the limited clinical specimens (typhi bloodstream infections) occurring here in Queensland through collaboration with Pathology Queensland.
- Refinement of assays (if warranted) to improve sensitivity and specificity.
- **Screening of clinical specimens in Lahore, Pakistan to validate assays in a XDR Typhoid-endemic setting**.
- Potential to validate and implement assays for use in Pakistan
- Gauge interest in pre-commercial validation with industry partners



Map of the Punjab province of Pakistan, with the capital Lahore located in the north-east.
Source: freeworldmaps.net

Disclosures

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