# CULTURE-INDEPENDENT DETECTION OF HELICOBACTER PYLORI ANTIMICROBIAL RESISTANCE MARKERS IN STOOL BY METAGENOMIC SEQUENCING

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### INTRODUCTION



Antimicrobial resistance (AMR) is increasingly common in *H. pylori* and contributes to treatment failure

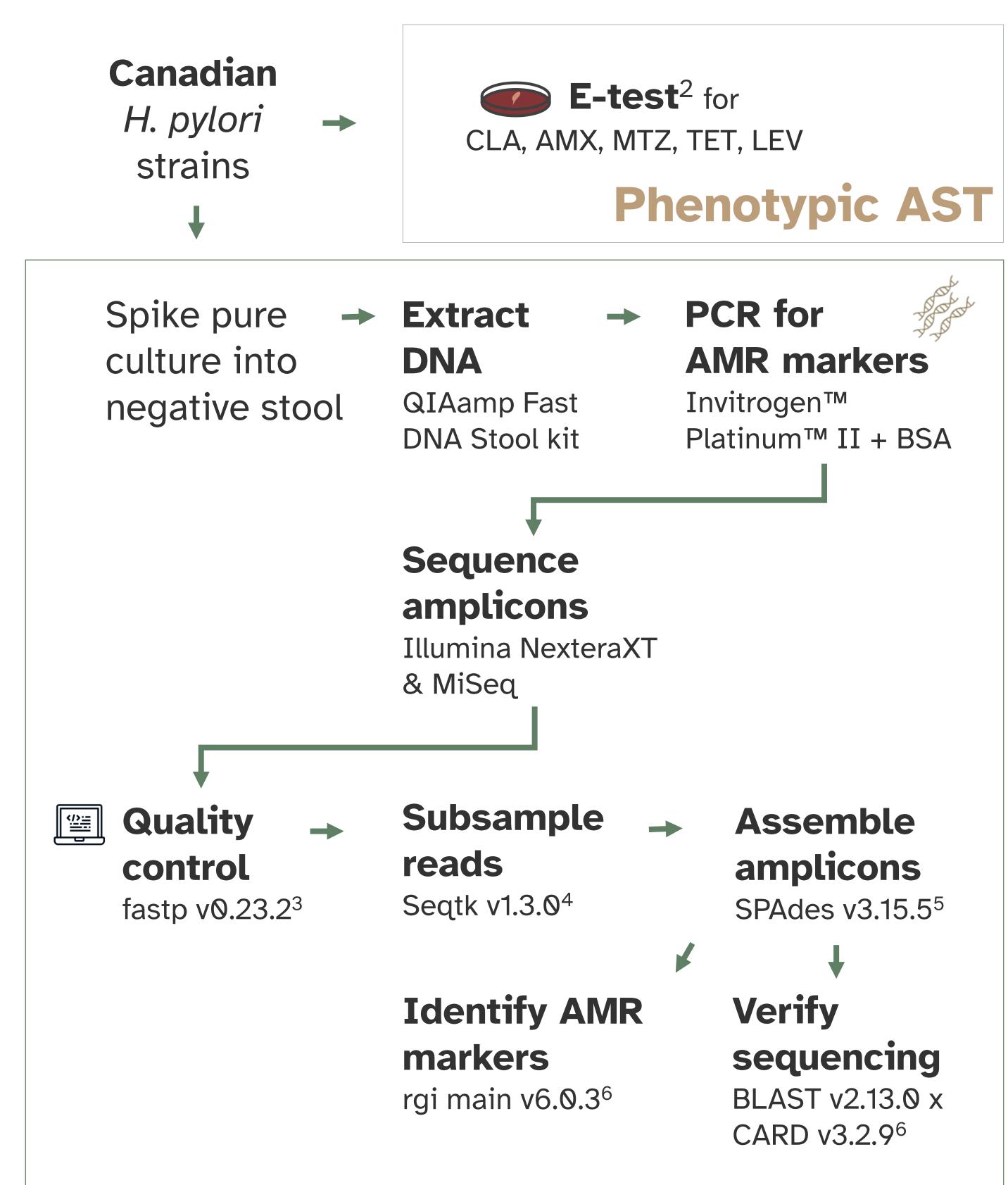


H. pylori is challenging to culture and phenotypic antimicrobial susceptibility testing (AST) is not routinely performed<sup>1</sup>



Sequencing could identify **AMR markers without culture**, support antibiotic stewardship and culture, support antibiotic stewardship, and improve treatment

### **METHODS**



## Genotypic AMR

Figure 1. Phenotypic AST was performed with E-tests®. Genotyping AST was performed using targeted-amplicon sequencing of AMR markers.

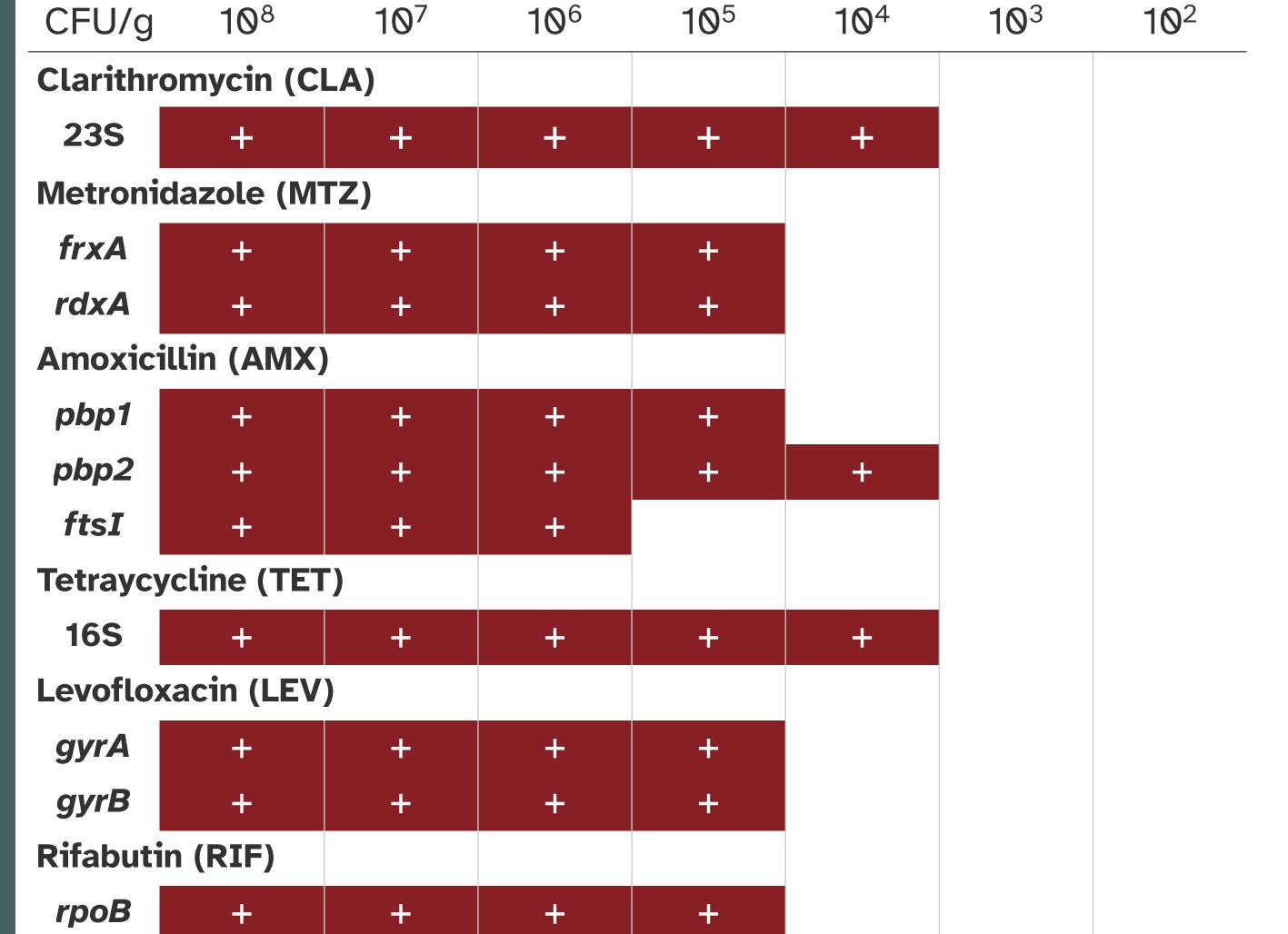
## **NEXT STEPS**

- Increase sensitivity in stool by comparing and optimizing DNA extraction methods
- Improve prediction of phenotypic AMR by applying machine learning classification
- Characterize genotypic AMR marker patterns typical of phenotypic resistance in Canadian isolates.
- Generate antibiogram for Canadian H. pylori isolates

## MAIN FINDING

H. pylori AMR markers can be detected from stool specimens without culture through targeted-amplicon sequencing.

**Table 1**. PCR amplification of *H. pylori* AMR determining genes produce visible bands (+) at 10<sup>6</sup> to 10<sup>4</sup> CFU/g in stool.



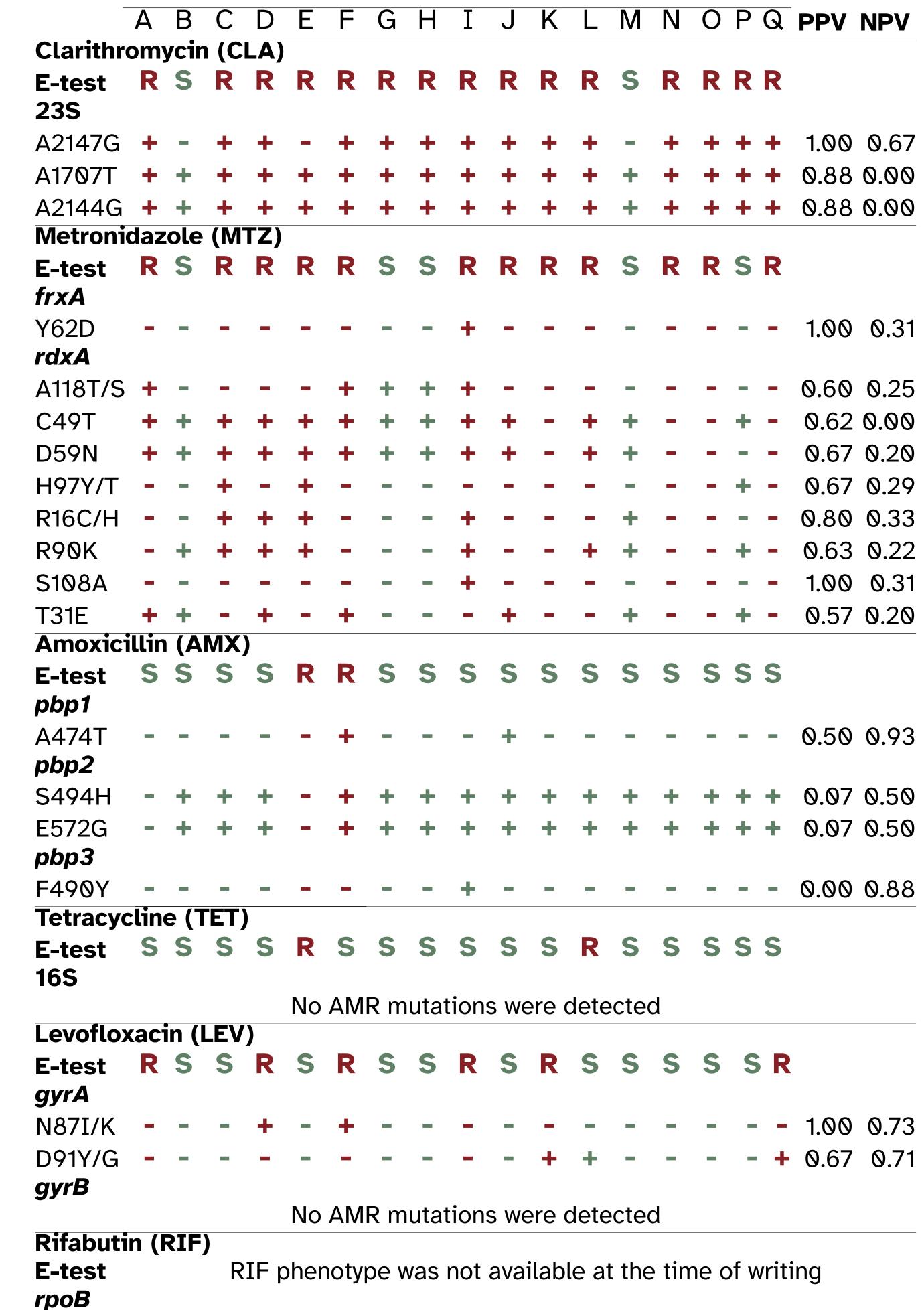
H. pylori strain ATCC 43504 was spiked into stool at concentrations of 108 to 102 CFU/g. PCR for AMR markers was performed on total DNA extracts and visualized by agarose gel electrophoresis.

## RESULTS

- Targeted-amplicon sequencing does not require isolation of *H. pylori*
- AMR markers for *H. pylori* can be detected in spiked stool specimens at spike-in concentrations of 10<sup>6</sup> to 10<sup>4</sup> CFU/g
- BLAST verified amplicon sequences were specific to the *H. pylori* and to the genes targeted
- Overall, genotypic AMR markers from global literature were detected in both phenotypically resistant and phenotypically susceptible Canadian isolates with PPV [0.00,1.00] and NPV [0.00,0.93]

**Table 2**. Detected (+) genotypic AMR markers vary in positive predictive value (PPV) for phenotypic AST (E-test) in sensitive (S) and resistant (R) strains (A to Q, n=17). PPV = P(R | +); NPV = P(S | -)

Isolate



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