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

Kaye Quizon[a], Matthew Walker[a], Shannon HC Eagle[a], Sara Christianson[a], Jillian Rumore[a], Natalie Knox[a], David Alexander[b,c], Jessica Forbes[d-f], Jessica Minion[g], Andrew G McArthur[h], Charles Bernstein[c,i], Seth R Shaffer[c,i], Aleisha Reimer[a]

[a]Public Health Agency of Canada, [b]Cadham Provincial Laboratory, [c]University of Manitoba, [d]Eastern Ontario Regional Laboratory Association, [e]University of Ottawa, [f]The Ottawa Hospital Research Institute, [g]Roy Romanow Provincial Laboratory, [h]McMaster University, [i]IBD Clinical and Research Centre

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[1]

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

METHODS

Canadian *H. pylori* strains → **E-test**[2] for CLA, AMX, MTZ, TET, LEV → **Phenotypic AST**

Spike pure culture into negative stool → **Extract DNA** QIAamp Fast DNA Stool kit → **PCR for AMR markers** Invitrogen™ Platinum™ II + BSA → **Sequence amplicons** Illumina NexteraXT & MiSeq → **Quality control** fastp v0.23.2[3] → **Subsample reads** Seqtk v1.3.0[4] → **Assemble amplicons** SPAdes v3.15.5[5] → **Identify AMR markers** rgi main v6.0.3[6] → **Verify sequencing** BLAST v2.13.0 x CARD v3.2.9[6]

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 10^6 to 10^4 CFU/g in stool.

CFU/g	10^8	10^7	10^6	10^5	10^4	10^3	10^2
Clarithromycin (CLA)							
23S	+	+	+	+	+		
Metronidazole (MTZ)							
frxA	+	+	+	+			
rdxA	+	+	+	+			
Amoxicillin (AMX)							
pbp1	+	+	+	+			
pbp2	+	+	+	+	+		
ftsI	+	+	+				
Tetraycycline (TET)							
16S	+	+	+	+	+		
Levofloxacin (LEV)							
gyrA	+	+	+	+			
gyrB	+	+	+	+			
Rifabutin (RIF)							
rpoB	+	+	+	+			

H. pylori strain ATCC 43504 was spiked into stool at concentrations of 10^8 to 10^2 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 10^6 to 10^4 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 A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	PPV	NPV
Clarithromycin (CLA)																			
E-test	R	S	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R		
23S																			
A2147G	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	1.00	0.67
A1707T	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.88	0.00
A2144G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.88	0.00
Metronidazole (MTZ)																			
E-test	R	S	R	R	R	R	S	S	R	R	R	R	S	R	R	S	R		
frxA																			
Y62D	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	1.00	0.31
rdxA																			
A118T/S	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	0.60	0.25
C49T	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	-	0.62	0.00
D59N	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	0.67	0.20
H97Y/T	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	0.67	0.29
R16C/H	-	-	+	+	+	-	-	-	+	-	-	-	+	-	-	-	-	0.80	0.33
R90K	-	+	+	+	+	-	-	-	+	-	-	+	+	-	-	+	-	0.63	0.22
S108A	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	1.00	0.31
T31E	+	+	-	+	-	+	-	-	-	+	-	-	+	-	-	+	-	0.57	0.20
Amoxicillin (AMX)																			
E-test	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S		
pbp1																			
A474T	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	0.50	0.93
pbp2																			
S494H	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	0.07	0.50
E572G	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	0.07	0.50
pbp3																			
F490Y	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	0.00	0.88
Tetracycline (TET)																			
E-test	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S		
16S	No AMR mutations were detected																		
Levofloxacin (LEV)																			
E-test	R	S	S	R	S	R	S	S	R	S	R	S	S	S	S	S	R		
gyrA																			
N87I/K	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	1.00	0.73
D91Y/G	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	0.67	0.71
gyrB	No AMR mutations were detected																		
Rifabutin (RIF)																			
E-test	RIF phenotype was not available at the time of writing																		
rpoB																			
I837V	-	-	-	+	-	-	+	+	-	-	+	-	-	-	+	-	-		
K2068R	-	+	-	+	-	-	-	-	-	-	+	+	+	-	+	-	-		
Q2079K	-	+	-	+	-	-	+	+	-	-	+	+	-	-	-	-	+		

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