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Point Mutations in Domain V of the 23S rRNA Gene are the Primary Cause of Clarithromycin Resistance in Clinical *Helicobacter pylori* Isolates in the United States

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Abstract

Background

Resistance to clarithromycin in *H. pylori* has been reported in several countries to be caused by specific point mutations in the 23S rRNA gene, particularly at positions 2143 and 2142 of domain V. Studies comparing these mutations in United States clinical isolates to antimicrobial susceptibility results determined using the gold standard agar dilution method are lacking. The purpose of this study was to assess for these point mutations in clinical *H. pylori* isolates in the United States, and to determine their ability to predict phenotypic antimicrobial susceptibility.

Methods

Archived *H. pylori* isolates previously submitted to the clinical microbiology laboratory at Mayo Clinic for antimicrobial susceptibility testing (AST) were retrieved. AST had been performed by agar dilution according to CLSI guidelines. The isolates were thawed, cultured on Columbia agar under microaerophilic conditions at 35ºC, and then lysed using bead beating. A 150 bp segment of the 23S rRNA gene was amplified via polymerase chain reaction and subsequently bidirectionally sequenced using dye-terminator technology. The presence of mutations at the 2143 and/or 2142 positions was recorded and compared to prior AST results.

Results

A total of 118 *H. pylori* isolates were tested. 7 isolates yielded insufficient *H. pylori* DNA for sequencing or did not have prior clarithromycin AST performed and were excluded from further analysis. For the remaining 111 isolates, there was concordance between phenotypic AST and genotypic results in 106 (95%) isolates. Of these, 21 (20%) were susceptible to clarithromycin and 85 (80%) were resistant. An A2143G mutation was identified in 70 (82%) isolates, A2142G in 12 (14%), and A2142C in 3 (4%). There was discordance between AST and genotype in 5 (5%) isolates; 3 had a resistant phenotype but a wild-type sequence, and 2 had a susceptible phenotype but an A2143G mutation.

Conclusions

23S rRNA gene mutations predict phenotypic resistance to clarithromycin in clinical *H. pylori* isolates. Resistance was most commonly conferred by A2143G, followed by A2142G and A2142C point mutations. The prevalence of each mutation in the United States is similar to that reported from other parts of the world.

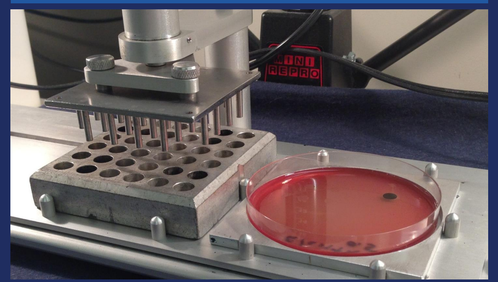
Objectives

- Characterize the distribution of 23S rRNA gene point mutations in clinical *H. pylori* isolates from the United States
- Determine the ability of a 150 bp segment of the 23S rRNA gene sequence to predict susceptibility to clarithromycin

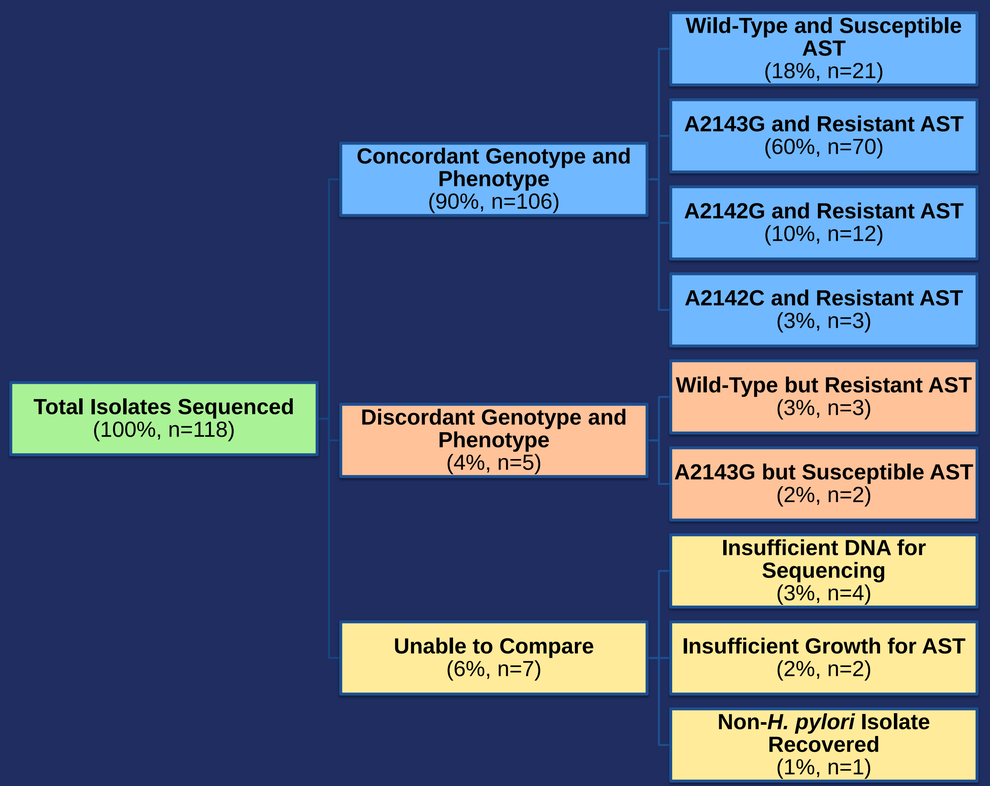
Methods

- Performed antimicrobial susceptibility testing using agar dilution on clinical *H. pylori* isolates submitted between November 2011 to October 2015
- Subcultured 118 archived *H. pylori* isolates
- Amplified and bidirectionally sequenced a 150 bp segment of the 23S rRNA gene
- Compared AST to sequence results

Agar Dilution



Results



150 bp Segment of 23S rRNA gene

5’-GAGCTGTCTCAACCAGAGATTC
AGTGAAATTGTAGTGGAGGTGAAAA
TTCCTCCTACCCGCGGCAAGACGG
AAAGACCCCGTGGACCTTTACTAC
AACTTAGCACTGCTAATGGGAATAT
CATGCGCAGGATAGGTGGGAGGCT
TTGAAG-3’

BLUE: forward and reverse primer locations
RED: 2142 and 2143 positions

Conclusions

- The distribution of 23S rRNA gene point mutations from clinical *H. pylori* isolates in the United States is similar to that reported from other parts of the world
- A2143G was the most common mutation, followed by A2142G and A2142C
- 23S rRNA gene sequence reliably predicted phenotypic susceptibility to clarithromycin