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Molecular Detection of Clarithromycin Resistance in Gastric Biopsies and Stool Samples from Patients with *Helicobacter pylori* Infection: A ReviewCarlos Herrera^{1*}, Yuki Nakamura² and Fatima Al-Zahrani³¹Department of Clinical Microbiology, University of Barcelona, Barcelona, Spain²Department of Gastroenterology, Kyoto University, Kyoto, Japan³Department of Infectious Diseases, King Saud University, Riyadh, Saudi Arabia

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Abstract

Clarithromycin resistance is the single strongest predictor of eradication failure in *Helicobacter pylori* infection, and the conventional way of measuring it, culture with phenotypic susceptibility testing, requires endoscopy and often fails to grow the organism at all. Molecular detection of the responsible point mutations avoids both problems, and can be done on stool.

Objectives: To review the published evidence on molecular detection of clarithromycin resistance in *Helicobacter pylori*: which mutations are targeted and how often each occurs, how molecular testing on gastric biopsies compares with culture, how stool-based testing performs against biopsy-derived reference standards, and what susceptibility-guided treatment achieves.

Material and Methods: Narrative review of diagnostic accuracy studies, assay evaluations, meta-analyses, consensus reports and guidelines indexed in PubMed and the major gastroenterology and clinical microbiology journals. Studies were eligible if they reported the prevalence or distribution of resistance-conferring mutations, the performance of a molecular assay against culture or sequencing, the accuracy of stool-based testing, or treatment outcomes stratified by resistance. No new patient data were generated; every frequency and accuracy estimate quoted is one published by the original investigators.

Results: Pooled across World Health Organization regions, primary and secondary clarithromycin resistance exceeded 15 per cent in all regions except the Americas and South-East Asia, and resistance was strongly associated with failure of clarithromycin-containing regimens, with an odds ratio of 6.97 (95% CI 5.23-9.28). In a prospective study of 1,200 patients, 23S rRNA mutations were found in 17.5 per cent of PCR-positive gastric biopsies, distributed as A2143G in 71.4 per cent, A2142G in 25 per cent and A2142C in 3.6 per cent; mixed susceptible and resistant infections were present in 1.9 per cent and were invisible to phenotypic testing. Pooling stool studies, PCR for clarithromycin resistance gave a sensitivity of 0.91 and a specificity of 0.97, with an area under the curve of 0.94.

Conclusion: Molecular testing detects resistance more reliably than culture, identifies mixed infections that phenotypic testing misses, and works on stool at accuracy adequate for clinical use. Its principal limitation is what it cannot see: it reports the mutations it is designed to detect and nothing else, so a negative molecular result excludes the common mechanisms rather than excluding resistance.

Abbreviations: *H. pylori* – *Helicobacter pylori*, CLR – Clarithromycin, MIC – Minimum Inhibitory Concentration, PCR – Polymerase Chain Reaction, RT-PCR – Real-Time Polymerase Chain Reaction, FRET – Fluorescence Resonance Energy Transfer, FISH – Fluorescence In Situ Hybridisation, rRNA – Ribosomal Ribonucleic Acid, WHO – World Health Organization, PPI – Proton Pump Inhibitor, OR – Odds Ratio, AUC – Area Under the Curve, FFPE – Formalin-Fixed Paraffin-Embedded.

Keywords: *Helicobacter pylori*; Clarithromycin Resistance; 23S rRNA; Stool PCR; Antimicrobial Stewardship

Introduction

Helicobacter pylori infects a large share of the world's population, with prevalence varying widely between countries and falling over time in high-income settings [1,2]. It is now formally classified as an infectious disease, which in principle means every infected patient should be treated [3,4]. It causes gastritis, peptic ulceration and

gastric adenocarcinoma, and eradication is the principal preventive intervention available for the last of these [5,6,7].

Treatment is where the difficulty lies. Clarithromycin has been the backbone of triple therapy because of its low minimum inhibitory concentration and good mucosal diffusion, and its widespread use in respiratory and paediatric practice has driven resistance upward [8,9]. In 2017 the World Health Organization

designated clarithromycin-resistant *H. pylori* a high priority for antibiotic research and development [8].

The mechanism is unusually tractable. Resistance is conferred almost entirely by point mutations in the V domain of the 23S ribosomal RNA gene, principally A2143G, A2142G and A2142C, which reduce macrolide binding to the peptidyl transferase region [10,11]. Because a small number of mutations account for most resistance, a targeted molecular assay can substitute for phenotypic susceptibility testing in a way that is not possible for most organisms [12,13].

That substitution matters practically because culture is a poor test. The organism is fastidious, sensitivity of culture from gastric biopsies has been reported as low as around half, and even when it grows, phenotypic testing is not always feasible [12,14]. Culture also requires endoscopy, which is a substantial constraint on testing everyone before treatment [15]. The aim of this review is to set out what molecular detection achieves on gastric biopsies and on stool, and what follows for how eradication therapy should be chosen.

Material and Methods

Search strategy: PubMed and the archives of the major gastroenterology and clinical microbiology journals were searched for studies of molecular detection of clarithromycin resistance in *Helicobacter pylori*. Search terms combined *Helicobacter pylori* and clarithromycin resistance with 23S rRNA, A2143G, A2142G, point mutation, real-time PCR, gastric biopsy, stool, faecal, susceptibility-guided therapy and tailored therapy. Reference lists of retrieved consensus reports and meta-analyses were screened by hand.

Eligibility: Studies were included if they reported the prevalence or distribution of resistance-conferring mutations in a defined population; the analytical or clinical performance of a molecular assay against culture, sequencing or another molecular reference; the accuracy of stool-based molecular testing; or eradication outcomes stratified by resistance status or by testing strategy. Consensus reports and guidelines were included where they define current recommended practice.

Data extraction and handling: Design, sample size, specimen type, assay platform, reference standard and reported accuracy estimates with confidence intervals were extracted as published. Reference standard is reported alongside every accuracy figure, because comparing a molecular assay against culture and against sequencing gives systematically different answers. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 3 and Tables 1 and 2 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

How common resistance is, and what it costs: A systematic review and meta-analysis grouped resistance estimates by World Health Organization region. Primary and secondary resistance to clarithromycin, metronidazole and levofloxacin exceeded 15 per cent in every region except the Americas and South-East Asia, where primary clarithromycin resistance was 10 per cent (95% CI 4-16). Fifteen per cent is the conventional threshold above which empirical clarithromycin-based regimens are discouraged [8].

The consequence for treatment is direct and large. Clarithromycin resistance was significantly associated with failure of clarithromycin-containing regimens, with an odds ratio of 6.97 (95% CI 5.23-9.28, $p < 0.001$) (Figure 3) [8]. The equivalent effect for

metronidazole is smaller, because metronidazole resistance can be partly overcome by increasing dose and duration, particularly within a bismuth-containing quadruple regimen [8,9]. Clarithromycin resistance cannot be overcome that way, which is why detecting it changes the regimen rather than the dose.

Which mutations, and in what proportion: In a prospective multicentre study of 1,200 patients undergoing endoscopy who were naive to eradication treatment, 1,086 gastric biopsies were tested. Culture was positive in 154 (14.2 per cent), of which 28 (18.2 per cent) were clarithromycin resistant by Etest. Molecular testing detected the organism in 160 (14.7 per cent), and among those, 23S rRNA resistance-conferring mutations were present in 28 (17.5 per cent) [16].

The distribution was A2143G in 20 cases (71.4 per cent), A2142G in 7 (25 per cent) and A2142C in 1 (3.6 per cent) (Figure 1) [16]. Comparable distributions have been reported elsewhere, with A2143G consistently dominant [10,17]. Additional mutations including T2182C and C2195T have been described, and their contribution to the resistant phenotype is less well established [17].

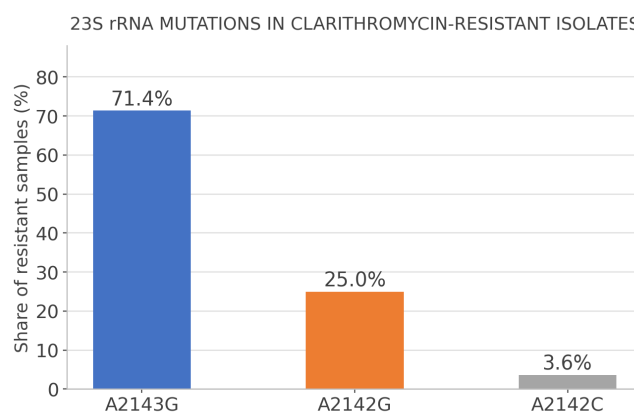


Figure 1: Distribution of 23S rRNA mutations among clarithromycin-resistant gastric biopsy samples [16]

What molecular testing sees that culture does not: The same study found mixed infections, containing both susceptible and resistant isolates, in 3 of 160 samples (1.9 per cent). In those cases phenotypic susceptibility testing detected only one population, so the mixed infection was invisible to Etest [16]. This is a structural advantage of molecular methods rather than an incidental one: they detect low relative amounts of resistant organisms within a mixed population, which culture by its nature cannot [12].

Molecular assays also work on material that culture cannot use, including formalin-fixed paraffin-embedded biopsies and gastric brushings [11]. Evaluations of commercial kits against culture and in-house PCR on gastric biopsies have reported sensitivity of 100 per cent with specificity of 98 to 99 per cent against the molecular comparator, and complete agreement on the resistance-conferring mutations identified [12,13].

Study	n	Specimen	Principal reported finding
Noguchi et al. 2007 [17]	—	Stool	Nested PCR detects mixed resistant and susceptible strains
Savoldi et al. 2018 [8]	Pooled	—	Resistance raises odds of treatment failure sevenfold
Hays et al. 2019 [12]	—	Biopsy	Commercial assay evaluated against culture and in-house PCR
Pichon et al. 2020 [16]	1,200	Both	Stool assay 96.3% sensitive; mixed infections missed by Etest
Gong et al. 2021 [18]	Pooled	Stool	Pooled sensitivity 0.91, specificity 0.97, AUC 0.94
Ren et al. 2023 [19]	Pooled	Stool	Individualised therapy guided by stool gene detection

Table 1: Principal studies reviewed, with specimen type and the finding as reported by the original investigators

Moving the test to stool: If resistance can be genotyped without endoscopy, the whole pathway changes. The prospective study above evaluated a commercial real-time PCR assay on stool DNA against culture with Etest and against quadruplex real-time PCR on paired antral and corpus biopsies. Sensitivity and specificity for detecting the organism in stool were 96.3 per cent (95% CI 92-98) and 98.7 per cent (95% CI 97-99) [16].

For resistance specifically, a meta-analysis of stool PCR studies reported a pooled sensitivity of 0.91 (95% CI 0.83-0.95) and pooled specificity of 0.97 (95% CI 0.62-1.00), with an area under the curve of 0.94 and a diagnostic odds ratio of 347.68 (Figure 2) [18]. A more recent pooling of eleven prospective studies covering 866 patients reported higher figures still, and subgroup analysis by PCR technique showed no notable variation [21,22].

The wide confidence interval around pooled specificity is worth pausing on, and the investigators attributed the underlying heterogeneity to pre-analytical factors: improper storage or transport and repeated freeze-thaw degrade stool DNA, and the abundant and diverse commensal flora in faeces can compromise specificity [18]. These are solvable handling problems rather than limitations of the principle, but they mean that performance in a well-run trial should not be assumed to transfer to routine practice without attention to sample handling [17,20].

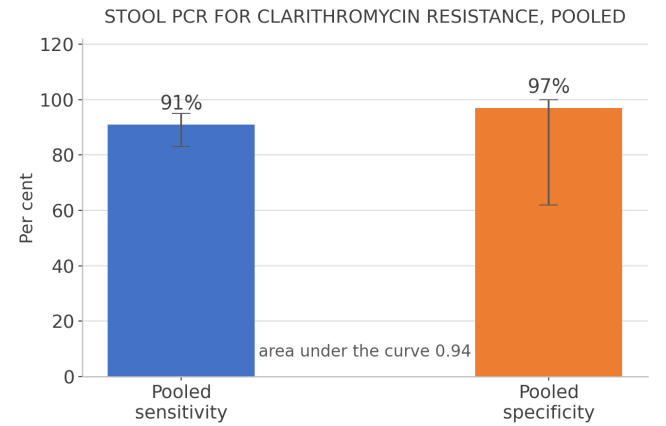


Figure 2: Pooled accuracy of stool PCR for detecting clarithromycin resistance, with 95 per cent confidence intervals [20]

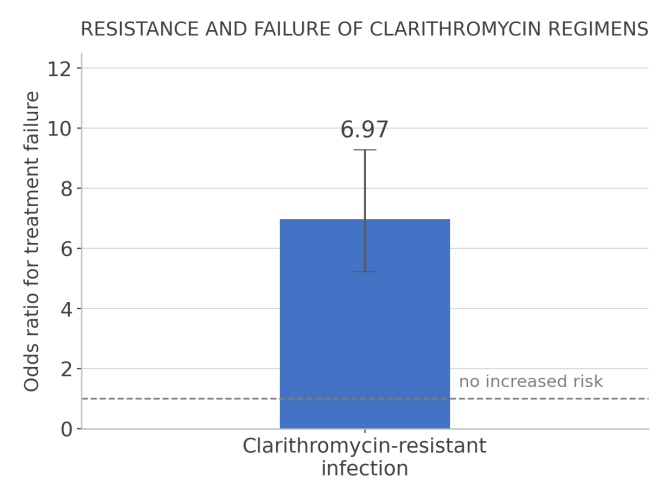


Figure 3: Association between clarithromycin resistance and failure of clarithromycin-containing eradication regimens [8]

From testing to treatment: Consensus guidance has moved steadily toward susceptibility-informed prescribing. The current European consensus recommends against empirical clarithromycin-based triple therapy where local resistance exceeds the threshold, favours bismuth quadruple or concomitant regimens, and supports antimicrobial stewardship through susceptibility testing where available [4,14]. Molecular detection is explicitly endorsed as an alternative when culture-based testing is not available [3,4].

Whether stool-guided individualisation improves eradication rates has been examined directly, with pooled analysis of individualised diagnosis and eradication therapy based on gene detection of clarithromycin resistance in stool specimens [21]. Practical assays continue to broaden, including detection from faecal specimens in young adults [20], stool antigen test reagents adapted to report resistance [21], and multiplex real-time platforms reporting both organism and genotype in a single run [11,13].

Approach	What it detects	Principal limitation
Culture with Etest	Phenotypic resistance to any agent	Requires endoscopy; low culture yield; misses mixed infection [14,16]
Biopsy molecular testing	Targeted 23S rRNA mutations	Reports only the mutations assayed; still requires endoscopy [12,13]
Stool molecular testing	Same mutations, no endoscopy	Sensitive to sample handling; heterogeneous specificity [16,20]
Empirical treatment	Nothing; assumes local resistance rates	Fails roughly sevenfold more often where resistance is present [8]

Table 2: Testing approaches, what each detects and the limitation reported for it

Discussion

The case for molecular testing here is stronger than for most biomarker substitutions, and the reason is biological rather than technical. Resistance is conferred by a handful of point mutations at known positions, so a targeted assay is not an imperfect proxy for the phenotype but a direct read of its cause [10,11]. That is why molecular testing outperforms culture rather than merely approximating it: it detects resistant subpopulations in mixed infections that phenotypic testing reports as susceptible [16], and it works on material culture cannot use [12].

The move to stool changes the pathway rather than the accuracy. Reported performance is broadly comparable to biopsy-based molecular testing [16,20,21], but the test can be done before any decision about endoscopy is made, which is what would allow susceptibility-informed prescribing to become routine rather than

exceptional [15,14].

What a negative result does and does not mean

The most important caveat is easily overlooked. A molecular assay reports the mutations it is designed to detect. A negative result therefore excludes A2143G, A2142G and A2142C, which between them account for the large majority of clarithromycin resistance [10,16], but it does not exclude resistance arising through less common mutations or through efflux mechanisms [10,11]. Phenotypic testing, whatever its practical failings, measures the property that matters directly.

In a population where the common mutations dominate, that residual is small and the trade is clearly worth making. Where a patient has already failed clarithromycin-based therapy and molecular testing is negative, the more informative next step is phenotypic testing rather than repeating the genotype [4,9].

Molecular testing of gastric biopsies in context

Resistance genotyping is one of several molecular assays now competing for the same endoscopic material. Eradication remains the principal modifiable intervention in gastric cancer prevention, and gastric cancer is still among the most commonly diagnosed malignancies worldwide [6,23,24]. Where cancer has already developed, the diagnostic biopsy must support testing for HER2, mismatch repair status, PD-L1 and claudin 18.2, each with its own tissue requirement [25].

Evidence from mismatch repair testing in this same setting shows that assay yield on small biopsy fragments is a genuine constraint, and that taking more fragments is a more useful response than running more assays on too few [26,27,30]. Expression-based markers vary appreciably between biopsy and resection material and between primary and metastatic sites [28,29,31]. Against that background, a stool-based resistance assay has a second and less obvious advantage: it removes the *Helicobacter pylori* question from the biopsy altogether, leaving the tissue for the assays that genuinely require it.

Study limitations

The limitations belong to the underlying literature. Pooled specificity for stool testing carries a very wide confidence interval, reflecting real heterogeneity in sample handling, DNA purification method, PCR platform, mutation set targeted and reference standard used [18]. Studies comparing molecular testing against culture and against sequencing are not directly comparable, since culture itself has limited sensitivity and molecular results discordant with culture are not necessarily false [11,16]. Several assay evaluations were conducted by or with the manufacturers of the platforms assessed [12,16]. Resistance prevalence varies enough between regions that figures from one setting should not be applied to another [8]. Reported stool assay performance may also be degraded by recent antimicrobial or acid-suppressant exposure, which has not been definitively assessed [11].

Two gaps deserve naming. No large randomised trial has compared stool-guided individualised therapy against current best empirical regimens with eradication as the primary endpoint, which is what would establish whether the testing pathway earns its cost [19]. And the clinical significance of mixed susceptible and resistant infections, detectable only by molecular methods, has not been quantified in terms of eradication outcome [16,17].

Conclusion

Clarithromycin resistance raises the odds of eradication failure roughly sevenfold and now exceeds the threshold for empirical use across most of the world. Because it is caused by a small number of identifiable point mutations, molecular detection is not a compromise substitute for culture but in several respects a better test: it detects mixed infections that phenotypic testing scores as susceptible, works on material culture cannot, and can be performed on stool with pooled sensitivity around 0.91 and specificity around 0.97. The honest limitation is scope rather than accuracy. A negative molecular result excludes the common mechanisms, not resistance itself, and in a patient who has already failed treatment that distinction should determine what is done next.

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Conflict of Interest

No competing interest is held by any of the authors.

Ethics Statement

Work already in the published record is all that this article examines. It collected no new patient data and recruited nobody, so ethical approval was never in question.

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