Journal of Global Antimicrobial Resistance 4 (2016) 35–41

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Review

Detection of clarithromycin-resistant *Helicobacter pylori* in clinical specimens by molecular methods: A review

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ARTICLE INFO

Article history:
Received 14 November 2015
Received in revised form 6 January 2016
Accepted 8 January 2016
Available online 8 February 2016

Keywords:
Helicobacter pylori
Clarithromycin resistance
Molecular methods

ABSTRACT

Various molecular methods have been developed to rapidly detect clarithromycin (CLR) resistance in *Helicobacter pylori* isolates in clinical specimens. All of these assays for detecting CLR resistance in *H. pylori* are based on detection of mutations in the 23S rRNA gene. In this article, we summarise current knowledge regarding the detection of *H. pylori* CLR resistance in clinical specimens by molecular tests. The available data showed that restriction fragment length polymorphism (RFLP), 3′-mismatch PCR, DNA sequencing, the PCR line probe assay (PCR-LiPA) and fluorescence in situ hybridisation assay (FISH) are able to detect CLR-resistant *H. pylori* in clinical specimens with excellent specificity and sensitivity. However, several factors limit their clinical application, including fastidious, time-consuming preparation and low-throughput as well as carrying a risk of contamination. Furthermore, as an invasive method, FISH is not suitable for children or the elderly. Among the molecular methods, one that is most promising for the future is real-time PCR probe hybridisation technology using fluorescence resonance energy transfer (FRET) probes, which can rapidly detect CLR resistance with high sensitivity and specificity in biopsies and stool specimens, even though mixed infections are present in clinical specimens. Moreover, due to the advantages that this method is simple, rapid and economical, real-time PCR is technically feasible for clinical application in small- and medium-sized hospitals in developing countries. Second, with high sensitivity, specificity and throughput, DNA chips will also be a valuable tool for detecting resistant *H. pylori* isolates from cultures and clinical specimens.

Contents

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http://dx.doi.org/10.1016/j.jgar.2016.01.002
2213-7165/

1. Introduction

Helicobacter pylori is a microaerophilic bacterium involved in digestive diseases such as peptic ulcer, gastritis and mucosa-associated lymphoid tissue (MALT) lymphoma and is a risk factor for the development of gastric cancer [1]. Infection with *H. pylori* is also a possible trigger for the development of cardiovascular disease [2]. Eradication of *H. pylori* infection has been shown to reduce the risk of recurrent peptic ulcer diseases as well as the development of gastric adenocarcinoma and MALT lymphoma [3].

Macrolide drugs such as clarithromycin (CLR) are a key component of first-line treatment to eradicate *H. pylori*. It is now widely accepted that CLR resistance among *H. pylori* strains has been increasing worldwide and is the most important factor responsible for the failure of *H. pylori* eradication [4,5]. To avoid resistance, culture and standard susceptibility tests have been recommended in regions or populations with high CLR resistance (≥15–20%) before the prescription of this first-line treatment [6]. Because of the slow growth and particular conditions required for *H. pylori* growth, culture represents a significant challenge [7]. In addition, agar disk diffusion or Etest methods for the determination of macrolide resistance require serial subcultivations for several days, and neither identify the type of point mutation present in the strain [8].

The mechanism leading to CLR resistance in *H. pylori* strains is well understood. CLR is a bacteriostatic antibiotic that binds reversibly to the peptidyl transferase loop of domain V of 23S rRNA, thus inhibiting protein synthesis in *H. pylori*. Resistance in *H. pylori* is mostly due to point mutations in the peptidyl transferase-encoding region of the 23S rRNA gene. Point mutations result in a decrease in affinity between ribosomes and CLR so that the drug is unable to interrupt protein biosynthesis [9]. However, the genotype distribution has geographical differences. Mutations A2143G and A2142G/C are most commonly associated with CLR resistance in natural *H. pylori* strains in Europe [10–17], Asia [18–22], South America [23,24], North America [25–27] and Africa [28,29], whilst A2144G [18,26,27,29,30] is also prevalent in some countries of Asia and North America. In addition, other 23S rRNA gene mutations, such as T2289C [31], C2245T [31], G2224A [31], T2182[12,22,32], T2717C [33] and T2243C [34], have been reported occasionally in macrolide-resistant *H. pylori* isolates.

Detection techniques for CLR resistance in *H. pylori* can be divided into two categories, standard susceptibility tests and molecular methods. Standard susceptibility tests are considered the 'gold standard' for confirmation of the resistance of *H. pylori*. However, isolation of *H. pylori* from gastric biopsies is affected by biopsy preparation and culture conditions. If standard susceptibility testing is not possible, molecular tests can be used to detect CLR resistance directly on gastric biopsies [6]. Molecular tests for detecting CLR resistance in *H. pylori* are based on detection of mutations in the 23S rRNA gene (Table 1). Currently, molecular methods, principally PCR-based assays, are becoming increasingly important, and various assays have been developed to detect CLR resistance in *H. pylori* isolates both in gastric biopsy samples and faeces. In this article, we summarise current knowledge regarding the detection of *H. pylori* CLR resistance in clinical specimens by molecular tests.

Table 1
Molecular methods for detection of point mutations associated with clarithromycin resistance in *Helicobacter pylori*.

PCR methods
Restriction fragment length polymorphism (RFLP)
3′-Mismatch PCR
DNA sequencing
DNA chips
Real-time PCR
PCR line probe assay (PCR-LiPA)
Non-PCR methods
Fluorescence in situ hybridisation (FISH)

2. Molecular methods for detecting clarithromycin resistance in *Helicobacter pylori*

2.1. Restriction fragment length polymorphism (RFLP)

RFLP analysis is a PCR-based assay traditionally used to detect point mutations in 23S rRNA from *H. pylori* strains directly from biopsy or faecal samples. To detect the A2143G and A2144G mutations, restriction digestion with *Mbo*II or *Bsa*I was performed on 23S rRNA PCR products amplified from gastric biopsy samples in a technique first described in 1997 [27]. In detail, point mutations were identified by amplification of the 23S rRNA target gene with subsequent digestion of the 1402-bp product to produce differently sized DNA fragments if mutations were present [27]. High-level resistance to CLR was more associated with the presence of the A2143G mutation than with the A2144G mutation in *H. pylori*. Since then, the PCR-RFLP method has frequently been used to detect mutations for CLR resistance, such as A2143G [18,22,23,27,35–46], A2142G [22,35–37,41–46] and A2144G [18,20,39]. If culture is accepted as the 'gold standard', the sensitivity and specificity of Scorpion PCR (real-time PCR) were 98.3% and 92.5%, respectively, and those of PCR-RFLP were 97% and 94%, respectively. Both Scorpion PCR and PCR-RFLP methods were superior to culture alone in detecting CLR mutations [37].

Over the past decade, a significant development has been made in the application of semi-nested or nested PCR-RFLP, not only for gastric biopsy samples but also for stool samples. In a report by Fontana et al. in 2003, semi-nested PCR combined with RFLP analysis was successfully applied for the detection of *H. pylori* CLR resistance in stool specimens [41]. Restriction analysis of 125 PCR products showed that only two samples contained CLR-resistant *H. pylori*, a result that was confirmed by antimicrobial susceptibility tests performed on *H. pylori* isolates obtained by culturing biopsy samples from the same patients [41]. Furthermore, other studies [42,43] have also demonstrated the usefulness of nested PCR-RFLP using stool samples to detect CLR-resistant *H. pylori*. Compared with the method invented by Fontana et al. [41], Rimbara et al. [43] improved the extraction method by extracting *H. pylori* DNA from faeces by enzymes and physical crushing using a modified Fast-Prep System and claimed that the new method was simpler and more efficient. Although they achieved >90% susceptibility by this nested PCR, the possibility of false-negatives was high. This was the main shortcoming of their study [43]. Unfortunately, it also has been difficult to demonstrate clear restriction patterns in the case of mixed infections using PCR-RFLP. Although the PCR-RFLP method is able to detect CLR-resistant *H. pylori* in clinical specimens with excellent specificity and sensitivity, several factors limit its clinical application, including the fact that it is low-throughput, cumbersome and carries a risk of contamination.

2.2. 3′-Mismatch PCR

Generally, PCR-RFLP allows the detection of only A-to-G mutations at base pair positions 2142, 2143 or 2144 of the 23S rRNA gene. However, the A2142C mutation has also been described for clinical isolates, although less frequently. Therefore, a method using 3′-mismatched PCR was developed using specific primers to identify the A2142C mutation in clinical isolates of *H. pylori* resistant to CLR. CLR primers 18 and 21 were used to amplify a 1400-bp fragment from an internal region of 23S rRNA followed by digestion with *Bsa*I and *Mbo*II. After using PCR-RFLP to detect the A2142G or A2143G mutations, CLR primers 18 and 3 were used for