Original Article

Genetic Diversity of *Helicobacter pylori* Isolates from Patients with Gastric Diseases in Isfahan

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Abstract

Background: *Helicobacter pylori (H. pylori)*, a spiral-shaped bacterium colonizing the human stomach, is generally acquired in childhood. This pathogen is highly diverse and can be used as genetic markers for predict the history of human migrations. This study aimed to determine the genetic diversity of *H. pylori* isolates from patients with dyspepsia by the multi-locus sequence typing (MLST) and update data on the prevalence of *H. pylori* among Iranian dyspeptic patients. **Materials and Methods:** In this descriptive cross-sectional study, 165 gastric biopsy specimens were obtained from patients with dyspepsia referred to Dr. Shariati Hospital of Isfahan, Iran, from April to July 2018. The status of *H. pylori* infection was determined by FISH in paraffin-embedded biopsy specimens. MLST of seven housekeeping genes was performed for 20 *H. pylori* isolates. The phylogenetic tree was plotted using CLC v8 and iTol software. **Results:** The overall prevalence of *H. pylori* infection was 53.3%. In the results of the analysis of MLST, a total of 14 new STs were recorded. The results of the global analysis showed that all the isolates, with a wide diversity, have a genetic affinity with members of the European population, such as Italy and Russia, and are in the hpEurope haplotype. **Conclusion:** Given the high prevalence of *H. pylori* infection in this region, early and accurate identification of patients seems necessary. Sequence analysis and determination of the origin of the phylogeny of strains can be effective in clinical management and monitoring of risk factors for chronic and recurrence of infection.

Keywords: *Helicobacter pylori, multilocus sequence typing, prevalence*

Introduction

Helicobacter pylori (H. pylori) is a Gram-negative spiral bacillus isolated from patients with chronic gastritis (CG) by Marshall and Warren in 1984. Infections caused by this bacterium are usually acquired in childhood and can remain asymptomatic for several years.[1] Extensive allelic diversity, genetic variability, mutations, and high recombination are prominent features of this microorganism.[2] *H. pylori* is naturally competent and can integrate a foreign DNA with homologous recombination into its genome. This feature allows the exchange of genetic matter between different strains living together in a stomach. Therefore, *H. pylori* is a panmictic species in which no diagnosable strong clonal structure exists.[3] Given that the microorganism has been probably in the human stomach for millions of years, it is considered to have evolved with its host. Therefore, it can be used as a reliable biological marker for the simultaneous evolution of host–pathogen and ancient human migration based on the diversity of sequences at the selected gene site.[4,5]

Genetic studies indicate that geographical and climatic contexts affect a high diversity of *H. pylori* strains. For instance, *H. pylori* strains of East Asia (Singapore and Korea) are distinct from European strains.[2,4] Besides, differences in gastric adenocarcinoma, chronic atrophic gastritis, and duodenal ulcers are partly associated with the geographical origins of strains.[6] Despite the high prevalence of infection in Africa and South Asia, the incidence of gastric cancer is much lower in these areas than in other countries, and the malignancies of this infection decrease from North to Southeast Asia. Therefore, genetic studies of *H. pylori* population are clinically important.[6,7]

H. pylori genotyping methods include multi-locus sequence typing (MLST), random amplification of polymorphic DNA,

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pulsed-field gel electrophoresis, whole-genome sequencing, amplified fragment length polymorphism, and others. MLST is a rapid and reliable method that based on allelic variation using the polymorphism of seven housekeeping genes has the advantages of high repeatability and high resolution and can provide more detailed information on human migration than human genetic analysis to a certain extent. In this technique, the data saved in the central database are accessible via the Internet and provides a potential source for global epidemiological study. However, MLST only reflects the variability of several housekeeping genes and the method is costly.[8,9]

H. pylori strains are divided into seven types based on their geographical associations: HpEurope, hpEastAsia, hpAfrica1, hpAfrica2, hpAsia2, hpNEAfrica, and hpSahul.[10,11] In a study performed in Iran by Farzi *et al*., the phylogenetic analyses showed that most of the strains fall into distinct clusters and were originally comparable to the ancestry of the hpEurope type.[4] It is estimated that 69% of Iran's population are now hosts of *H. pylori.* Therefore, the infection caused by this bacterium is a major concern in Iran.[12] Furthermore, Iran is a large country with multiple neighbors and more than 14 diverse ethnic groups and is at the intersection of Europe, Asia, and Africa and it appears that a diverse genetic population of *H. pylori* strains exists in this country. Studies on the analysis of *H. pylori* population in the capital of Iran (Tehran) also indicated various alleles in the strains.[13] However, there is no study on molecular typing of *H. pylori* strains in Isfahan Province, which is located in the center of Iran.

The present study aimed to determine the genetic diversity of *H. pylori* strains isolated from the adult population of Isfahan Province using the MLST method and update information on the prevalence of gastrointestinal infections caused by the bacterium.

Materials and Methods

Study design

A total of 165 patients with dyspeptic symptoms were referred to the Outpatient Gastroenterology Clinic and Endoscopy Unit in Dr. Shariati Hospital of Isfahan, Iran, as a participating center in the Global Antimicrobial Resistance Surveillance System Project of the World Health Organization. These patients underwent gastric endoscopy between April 2018 and July 2018. Patients with upper abdominal pain, dysphagia, nausea, vomiting, dyspepsia, and gastroesophageal reflux were included in this study. Patients who had received antibiotics, H2-receptor blockers, proton-pump inhibitors, and nonsteroidal anti-inflammatory drugs within 15 days prior to endoscopy were excluded from the study. Clinical findings and detailed epidemiological were determined for each patient. Two antrum and corpus biopsies were obtained from each patient. One set was fixed and transported in 10% buffered formalin for histopathological examination; a second set of biopsies was placed in sterile Eppendorf tubes, containing 1 ml sterile physiological solution (0.9%Nacl), and immediately transported to the microbiology laboratory for *H. pylori* culturing.[14,15]

Histology

The biopsy tissues in 10% formalin were processed for histopathology using an automated tissue processor (ATP). Formalin-fixed paraffin-embedded tissue blocks were sectioned using a microtome, cut into 3–4 μm sections, and dewaxed and used for histopathological staining. Slides were stained with H and E and Giemsa by routine protocols. All samples were evaluated independently by two pathologists.[14] In addition, cases of CG were graded according to the grading system provided by Houston-updated Sydney System which was depended on the severity of inflammatory cell infiltration in the lamina propria into four scales as follows:[16]

(i) Grade 0 – Absent inflammation
(ii) Grade 1 – Mild inflammation
(iii) Grade 2 – Moderate inflammation
(iv) Grade 3 – Severe inflammation.

Determination of *Helicobacter pylori* infection

H. pylori prevalence was determined by FISH. Briefly, for the hybridization of the samples, each slide of the tissue sections was overlaid with 50 μl of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl, pH 8, 0.01%SDS, and 20% formamide) containing 5 ng/μl of fluorescein isothiocyanate-labeled oligonucleotide Hpy-1.

Probe Hpy-1 (5-CACACCTGACTGACTATCCCG-3) targeted to a 16S rRNA position was used to specifically identify *H. pylori*.[14,17]

Culture

Biopsy specimens were sent to the clinical microbiology lab within half an hour of sampling in sterile tubes. Then, the specimens were homogenized in saline and inoculated on selective medium Columbia Agar (Gibco, USA) supplemented with 7% sheep blood and 10% fetal calf serum (FCS) and *H. pylori* selective supplement (DENT) (Oxoid). The plates were incubated for 5–10 days at 37°C in a microaerophilic environment (Anoxomat; MART Microbiology BV, Drachten, The Netherlands). *H. pylori* was identified based on colony morphology, Gram stain as a gull wing-shape bacteria, and also by positive reactions for oxidase, catalase, and strong urease activity. The strains were then stored in brain heart infusion broth with 30% glycerol and 7% FCS at −70°C until molecular typing was performed.[18]

Multi-locus sequence typing analysis

After the recovery of strains in liquid medium BHI and culturing on Columbia agar, one or more single colonies were isolated from each of 20 patients and used for MLST

analysis. The criterion for selecting isolates for MLST was pathology findings of patients. The characteristics of these strains are shown in Table 1.

Table 1: Characteristics of strains for phylogenetic analysis used in this study

Patient ID	Age (years)	Gender	Area	Pathology	*Cag* PAI
2633	42	Male	Esfahan	CG	+
2634	68	Male	Esfahan	CAG	+
2635	70	Female	Shahreza	CG	+
2636	48	Female	Esfahan	CG	–
2637	32	Male	Esfahan	EG	+
2638	76	Male	Esfahan	CAG	+
2639	56	Female	Flavarjan	EG	+
2640	40	Female	Esfahan	CG	–
2641	53	Male	Najaf Abad	EG	–
2642	44	Female	Khomeinishahr	CG	+
2643	28	Male	Shahreza	CAG	+
2644	65	Male	Zarrinshahr	EG	–
2645	59	Female	Mobarakeh	CAG	+
2646	75	Female	Esfahan	CAG	+
2647	66	Male	Khomeinishahr	CG	+
2648	50	Female	Esfahan	EG	–
2649	38	Male	Flavarjan	CAG	+
2650	61	Female	Esfahan	CAG	+
2651	77	Male	Shahreza	CG	+
2652	36	Female	Esfahan	EG	+

CG: Chronic gastritis, CAG: Chronic active gastritis, EG: Erosive gastritis, PAI: Pathogenicity island

DNA was extracted using a DNA extraction kit NucleoSpin Microbial (MACHEREY-NAGEL, Germany) according to the manufacturer's instructions**.** The isolates were further confirmed as *H. pylori* using PCR amplification of an *ureC* (*glmM*) fragment. PCR was performed as described by Nafisi *et al*.[19] The presence of the *cagA* gene was determined by *cagA* PCR. PCR conditions have been previously reported.[20] The primers are listed in Table 2.

For phylogeographic, typing the seven standard MLST genes (atpA, *efp*, *mutY*, *ppa*, *trpC*, *ureI*, and *yphC)* were amplified for each strain.[21] Primer pairs were purchased from Metabion, Munich, Germany [Table 3].[13] Each PCR product was purified and then sequenced by the Sanger method (micro synth-Swiss) using forward and reverse primers.

Phylogenetic analysis

All chromatograms were analyzed and edited using Chromas software (Version 2.6.5) Technelysium Pty Ltd (http://www.technelsium.com au). Then, the sequences were submitted to the *H. pylori* MLST database (http://pubmlst. org/Helicobacter) for allele and sequence type identification. The allele numbers assigned to each of the seven genes for each isolate were recorded in the MLST profile, and the sequence types for each isolate were determined. The phylogenetic three of 20 Iranian *H. pylori* isolates based on concatenated nucleotide sequences of 7 gene loci were constructed using the Neighbor-Joining algorithm implemented in CLC Sequence Viewer 8 (http://

Table 2: Primers used for the amplification of *Helicobacter pylori* genes

Target gene	Primers (5'-3')	Annealing temperature (°C)	Size of PCR products (bp)
ureC-F	TGG GAC TGA TGG CGT GAG GG	50	820
ureC-R	AAG GGC GTT TTT AGA TTT TT		
CagA-F	AATACACCAACGCCTCCAAG	55	400
CagA-R	TTGTTGGCGCTTGCTCTC		

PCR: Polymerase chain reaction

Table 3: Primer sequences used for polymerase chain reaction amplification and sequencing of the seven housekeeping genes for *Helicobacter pylori* and expected product sizes for each reaction

Target gene	Primers (5'-3')	Annealing temperature (°C)	Size of PCR products (bp)
trpC	F: TAGAATGCAAAAAAGCATCGCCCTC R: TAAGCCCGCACACTTTATTTTCGCC	58	633
yphC	F: CACTATTACCACGCCTATTTTTTTGAC R: TTTCTARGCTTTCTAAAATATC	56	734
atpA	F: GGACTAGCGTTAAACGCACG C: CTTGAAACCGACAAGCCCAC	56	841
mutY	F: GTGGTTGTAGYTGGAAACTTTACAC R: CTTAAGCGTGTGTYTTTCTAGG	58	676
efp	F: GGCAATTTGGATGAGCGAGCTC R: CTTCACCTTTTCAAGATACTC	56	559
ppa	F: GGAGTGAGCCATGACGCTGA R: GCTTTTATSGCTTTYTCTAAAAC	53	706
ureI	F: AGGTTATTCGTAAGGTGCG R: GTTTAAATCCCTTAGATTGCC	52	686

*http://pubmlst.org/Helicobacter. PCR: Polymerase chain reaction

www. clcbio. com). Then, 25 isolates were selected and a phylogenetic tree was created to compare Iranian isolates with Asian countries in iTol software (https://pubmlst. org/helicobacter/). To analyze the population structure and determine the relatedness of Iranian *H. pylori* to worldwide populations, 103 sequences from hpEurope, hpEastAsia, hpAfrica2, hspWAfrica1, hpSahul, hpNEAfrica, and hpAsia2 strains were selected. Bootstrap analysis was performed with 1000 replications, and Phylogenetic tree was edited by using iTol software.[22] Analyses were performed in SPSS version 20.

Results

Prevalence of *Helicobacter pylori* infection

A total of 165 patients, including 84 males and 81 females, enrolled in this cross-sectional study. The mean age was 50.3 ± 15.5 years old with the age ranging between 15 and 83 years. Eighty-eight of 165 patients (53.3%) were diagnosed as *H. pylori* positive by FISH. Among 88 *H. pylori* positive patients, 81 (49%) were positive by histology. By culture, 83 (50.3%) patients were positive. Agreement was found between the results of the diagnostic tests ($P < 0.001$).

Histopathological findings

In gastric biopsies, gastritis was found in 155 cases (94%), of which 86 (52.1%) patients were identified as CG, 36 (21.8%) as chronic active gastritis, 31 (18.8%) as erosive gastritis (EG), and 2 (1.2%) gastric cancer. Pathological changes such as intestinal metaplasia in 6 patients with CG were also observed. The most common type of inflammation was Grade 1 (43.6%). The results are shown in Figure 1. *H. pylori* colonization was seen in 81 biopsies.

Identified alleles and nucleotide diversity of the housekeeping genes

Among the 83 *H. pylori* strains recovered from cultured biopsy specimens, 20 strains were used for molecular typing. MLST showed that 77 new alleles were identified for the genes included in the scheme. These were distributed as follows: 13 new alleles for the gene *atpA*, 9 for *efp*, 11 for *mutY*, 11 for *ppa*, 13 for *trpC*, 7 for *ureI*, and 13 for *yhpC*. The highest allelic diversity was in the three genes, *trpC*, *atpA,* and *yphC* and the lowest in the *ureI* gene. In three of the 20 isolates (15%), all seven alleles sequenced were described for the first time. The allelic profile for each isolate indicates that each of the 20 sequence types is unique and not previously described in the MLST database [Table 4]. This new sequences were uploaded to the *H. pylori* Public MLST Database (http://pubmlst. org/helicobacter/) [Supplementary Files].

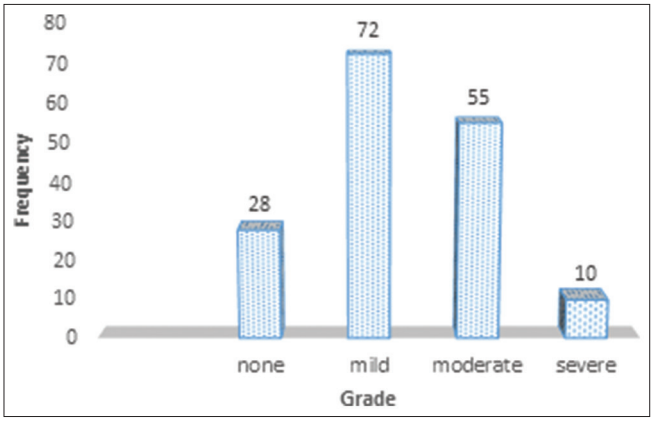

Figure 1: The frequency of grading the severity of inflammation of the samples studied, according to the Sydney System

Population structure and phylogenetic analysis

A phylogenetic tree was constructed based on sequence datasets obtained from the seven housekeeping genes of 20 Iranian *H. pylori* strains in software CLC Sequence Viewer 8. The isolates recorded in this study were clustered into three groups. In the first cluster, only one ST was observed, in the second cluster, 15 STs, and in a third of the cluster, 4 STs were observed [Figure 2]. The Simpson 'Diversity Index 77% was calculated.

In Figure 3, the isolates of this study were compared to those of the neighboring countries of Iran in the iTol software. The phylogenetic analyses revealed that 45% of the Iranian *H. pylori* strains were clustered together with the isolates from Turkey, Saudi Arabia, Palestine, and Lebanon. The other isolates were clustered into five other groups.

The results of the global analysis of the *H. pylori* sequences of different geographical regions and ethnic groups different from other populations and Iranian isolates indicated that

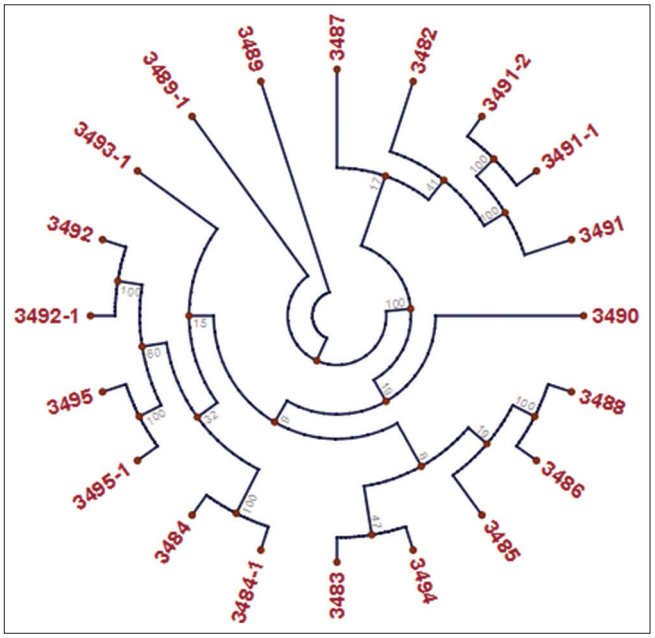

Figure 2: Analysis of MLST 20 Isolated Iranian *Helicobacter pylori*. Phylogenetic tree using neighbor-joining-tree algorithm in software Viewer 8 CLC Sequence been plotted

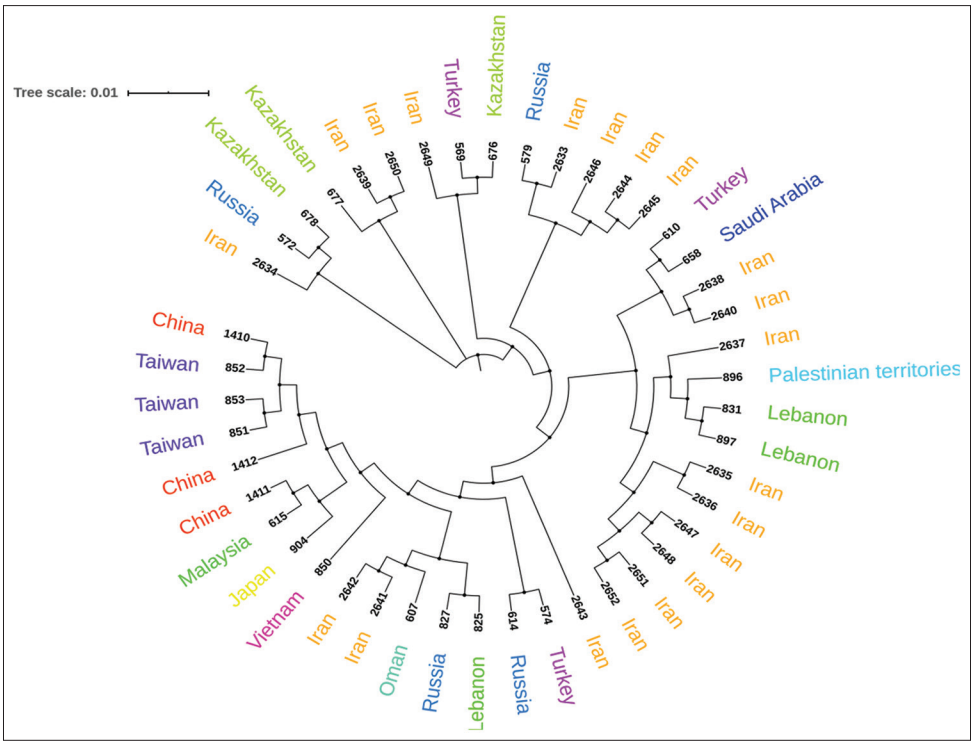

Figure 3: Phylogenetic analysis of 20 isolated Iranian *Helicobacter pylori* isolates with 25 isolates from neighboring Asian countries using data from 7 housekeeping genes in the iTol software

Table 4: The allelic profile for the isolates included in the study*

Number	ID	Isolate	Allele							ST
			atpA	*efp*	*mutY*	*ppa*	*trp*	*ureI*	*yphC*	
1	2633	ISF1	**2759**	1861	1672	**2636**	**2851**	**2859**	**2894**	3482
2	2634	ISF2	**2760**	**2584**	**2795**	**2637**	**2852**	118	**2895**	3483
3	2635	ISF3	**2761**	**2585**	**2796**	**2638**	**2853**	44	**2896**	3484
4	2636	ISF4	**2761**	**2585**	**2796**	**2638**	**2853**	44	**2896**	3484
5	2637	ISF5	**2758**	**2586**	1714	**2639**	**2855**	**2860**	**2897**	3485
6	2638	ISF6	**2763**	**2587**	**2797**	**2640**	**2856**	**2861**	**2897**	3486
7	2639	ISF7	**2764**	**2588**	**2798**	**2641**	**2857**	**2862**	**2900**	3487
8	2640	ISF8	**2765**	**2587**	**2799**	**2642**	**2858**	**2861**	**2901**	3488
9	2641	ISF9	**2768**	71	2801	**2645**	**2861**	1660	1705	3489
10	2642	ISF10	**2768**	71	2801	**2645**	**2861**	1660	1705	3489
11	2643	ISF11	1692	**2589**	**2800**	**2643**	**2859**	**2863**	**2902**	3490
12	2644	ISF12	**2767**	2506	1990	**2403**	**2860**	**2864**	**2903**	3491
13	2645	ISF13	**2767**	2506	1990	**2403**	**2860**	**2864**	**2903**	3491
14	2646	ISF14	**2767**	2506	1990	**2403**	**2860**	**2864**	**2903**	3491
15	2647	ISF15	**2770**	866	**2802**	**2646**	**2862**	2140	**2904**	3492
16	2648	ISF16	**2770**	866	**2802**	**2646**	**2862**	2140	**2904**	3492
17	2649	ISF17	**2771**	**2590**	**2803**	**2647**	**2863**	**2865**	**2905**	3493
18	2650	ISF18	**2772**	**2592**	**2804**	2577	2864	1774	**2906**	3494
19	2651	ISF19	**2773**	**2593**	**2805**	1367	1434	1698	**2907**	3495
20	2652	ISF20	**2773**	**2593**	**2805**	1367	1434	1698	**2907**	3495

*The numbers correspond to the identification of the alleles in the *H. pylori* public MLST database and the numbers in bold represent the alleles that were first described in this study. *H. pylori: Helicobacter pylori*, MLST: Multi-locus sequence typing

these strains were comparable to European ancestors and fell into the hpEurope haplotype containing isolates from Russia and Italy [Figure 4].

CagA is one important *H. pylori* virulence factor secreted by the type IV secretion pathway. Seventeen Isfahan strains (%85) were *cagA*-positive strains.

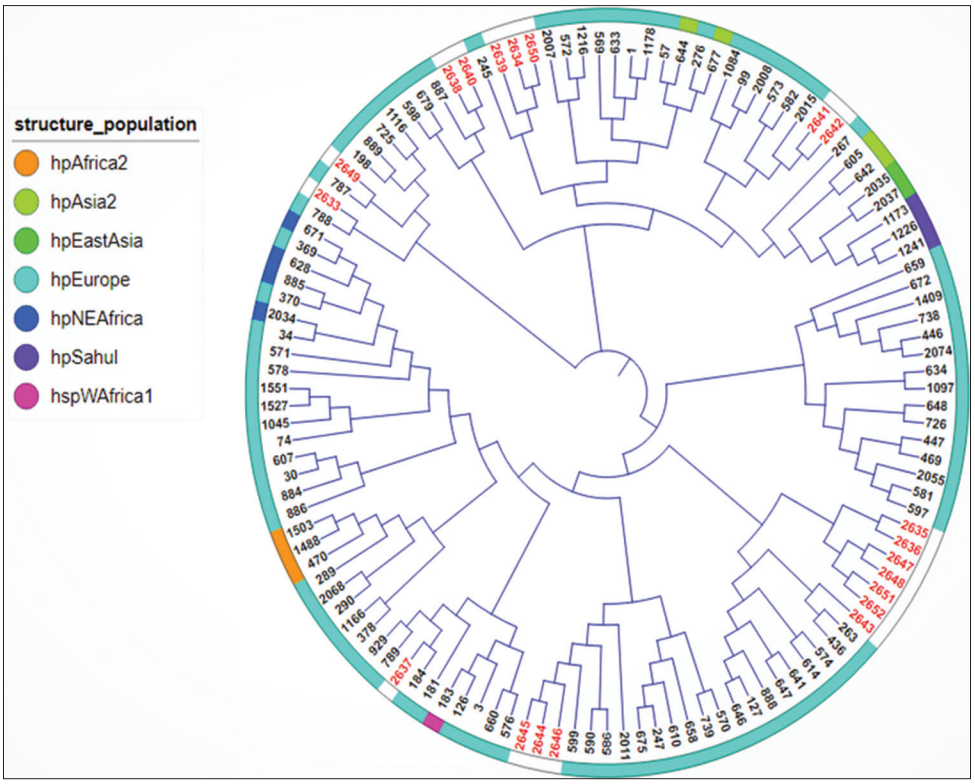

Figure 4: Global analysis of 20 Iranian isolates with103 strains from other countries using 7 housekeeping genes in the iTol Software based on population structure of isolates (*H. pylori* strains of Isfahan are red)

Discussion

H. pylori has been reported from all over the world, and it plays an important role in the development of upper gastrointestinal tract diseases.[23] In the present study, the prevalence of *H. pylori* in patients with gastrointestinal symptoms in Isfahan was 53.3%.

The prevalence of *H. pylori* infection in Iran is relatively high and varied in terms of geographical distribution, which is reported from 40% to 90% in different regions (south to north of Iran).[24] In one study conducted in western Iran, the prevalence of positive cases was reported to be 71% that was higher than previously published data.[25] Bayati *et al*. isolated *H. pylori* in biopsy specimens of 32% of participants by the culture method in Tehran.[26] Studies indicate the existence of various *H. pylori* infections in different countries. The highest rate belonged to Nigeria (89.7%), and the lowest rate was for Yemen (8.9%). In general, the infection was more common in developing countries (50.8%) than in developed countries (34.7%).[27] Various factors such as health and socioeconomic conditions and even the lack of effective methods for purification of drinking water in some regions as well as the transmission to humans due to the reuse of treated water for agricultural purposes, livestock and industry in arid and semi-arid areas in the world, like Iran, can be the justification for the disease at a younger age in this geographical region.[24] However, more research is needed to determine whether changes in the prevalence of *H. pylori* infection affect the global burden of upper gastrointestinal diseases.

Determination of the genetic characteristics of pathogenic microorganisms plays a crucial role in understanding the epidemiology of infectious diseases in the development of diseases.[28] Countries with ethnically diverse populations provide us with a good opportunity to learn more about the structure of *H. pylori* populations.[28,29] Iran is a vast country with diverse ethnic groups (a combination of ethnicities, including Kurds, Lors, Fars, Gilaks, Arabs, and Azeris) and is located at the intersection of Europe, Asia, and Africa. Ethnic and geographical diversity in Iran is a reflection of historical interaction with other populations. For instance, the Arab population of Iran entered the country in the 7th and 8th centuries AD during the victory of Islam over the Iranian Empire. The Kurdish population in northwestern Iran has historical ties with Turkish Kurds and other Turks throughout history, including the Ottoman Empire and World War I.[12] In the present study, there was a genetic similarity between *H. pylori* isolated from the Iranian population and similar geographical species in neighboring countries such as Turkey and Kazakhstan. Genetic similarity existed between isolates from Iran and the isolates in Palestine, Lebanon, Russia, and Saudi Arabia. The finding was consistent with one study conducted by Latifi-Navid *et al*. in Iran and could reflect continuous historical contacts over the years.[13]

The gastrointestinal infections caused by this bacterium in the incidence of gastric cancer, as the deadliest type of cancer in Iran, are estimated to be 75%–80%. It has been found that the frequency of gastric ulcers and cancer is considerably influenced by geographical conditions and ethnic groups.[5] Throughout history, Iran has had a population of diverse ethnic groups. Therefore, the probability of transmission of DNA between strains of different genotypes may be higher in the country than in other countries. In the present study, the genetic analysis of *H. pylori* by MLST indicated that strains had panmictic structures, and no clonal structure was observed. Horizontal gene transfers and repeated recombination can lead to extensive genetic rearrangement. *H. pylori* appears to have a clonal structure only in short periods, such as a change from one host to another. However, the evidence suggests that mutations may play roles in the genetic diversity of *H. pylori.*

In the present study, most alleles were first recorded in Pub MLST, and thus, the isolates had different STs probably due to the occurrence of extensive single-nucleotide polymorphisms, homologous recombination, and the emergence of pseudogenes through homopolynucleotide mutations in the genome. The MLST analysis indicated that the *ureI* gene was the most common allele among the Iranian *H. pylori*. In one study on the Malay population by Tai *et al*., *trpC* was the most diverse gene in all patients, except for the Chinese population of Malaysia with the highest diversity of approximately 7.6%, while the lowest gene diversity belonged to *atpA* with 2.6%.[30] On the contrary, based on the sequence analysis of MLST in the data set obtained from this study, three genes, *atpA, yphC,* and *trpC*, showed the greatest diversity than the other genes did.

In a study performed in Iran by Farzi *et al*., 38% of the Iranian *H. pylori* strains were similar in at least three gene loci and the *ureI* gene as the most identical allele among Iranian *H. pylori* strains.[4] The difference between *H. pylori* isolates may be for various reasons such as chromosome rearrangement, point mutations in protected genes, and the presence of unprotected genes like *cagA.*

de Sablet *et al*. conducted one study investigating the roles of phylogenetic origins of strains as determinants of gastric cancer risk in *cagA*-positive patients in two high-risk and low-risk regions for gastric cancer in Colombia. They found that hpEurope-dependent and hpAfrica1-dependent strains were in the high-risk and low-risk regions of stomach cancer, respectively. People infected with hpEurope strains had more advanced histological lesions and further damage to the DNA of the stomach epithelial cells. Therefore, the ancestral origin of strains was introduced as a strong predictor of gastric cancer risk.[7]

In this study, the relationship between the etiology of phylogeny and clinical results could not be examined owing to the small number of strains used in the analysis of MLST and the uncertainty of people with high risk and low risk of gastric cancer; however, a global analysis of 103 strains of the *H. pylori* reference from 7 phylogenetic populations indicated that strains isolated from Isfahan were in the hpEurope type population. Latifi-Navid *et al*. reported findings similar to our study results by evaluating the *H. pylori* species isolated from different geographical regions of Iran.[13] In the current study, among 20 isolates, 14 new STs not previously recorded in the MLST database were reported, demonstrating the high level of recombination between the isolated strains. In line with our study, Alvandi *et al*. suggested that the *H. pylori* populations have no clonal structure and all strains isolated from Kermanshah close to the population of Spain–Estonia belonging to the hpEurope.[12] Latifi-Navid *et al*. in 2010 reported that sequence types were close to strains isolated from Europe–Western Asia which confirms the findings of the current study.[13]

It is recommended that more extensive studies be conducted among different geographical populations in Iran to study the genetic diversity and find the relationship between the geographical origins of strains and the severity of *H. pylori* pathogenesis.

However, the typing of *H. pylori* population can be utilized as a useful tool to map human migration patterns and highlight the roles of ancestral origins in the development of gastric cancer.

Conclusion

The present study results indicated that the prevalence of *H. pylori* infection was high in patients with gastrointestinal symptoms in Isfahan. Therefore, it is important to adopt rapid, reliable, and cost-effective diagnostic methods in these geographical regions. Typing of the strains selected in the present study indicated that the isolates were in the hpEurope population. Determination of the phylogeny source of *H. pylori* strains may provide a useful strategy for clinical management, including the intensification of eradication programs and monitoring of the risk factors for gastric cancer caused by this infection.

Ethics approval

The study was approved by the Research Ethics Committee of the Isfahan University of Medical Sciences (No: IR. Mui. rec. 1396.3.878), Isfahan, Iran.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Files

Table 1: Register 3482ST on Pub multi-locus sequence typing site and confirm strain specification (http://pubmlst.org/helicobacter/)

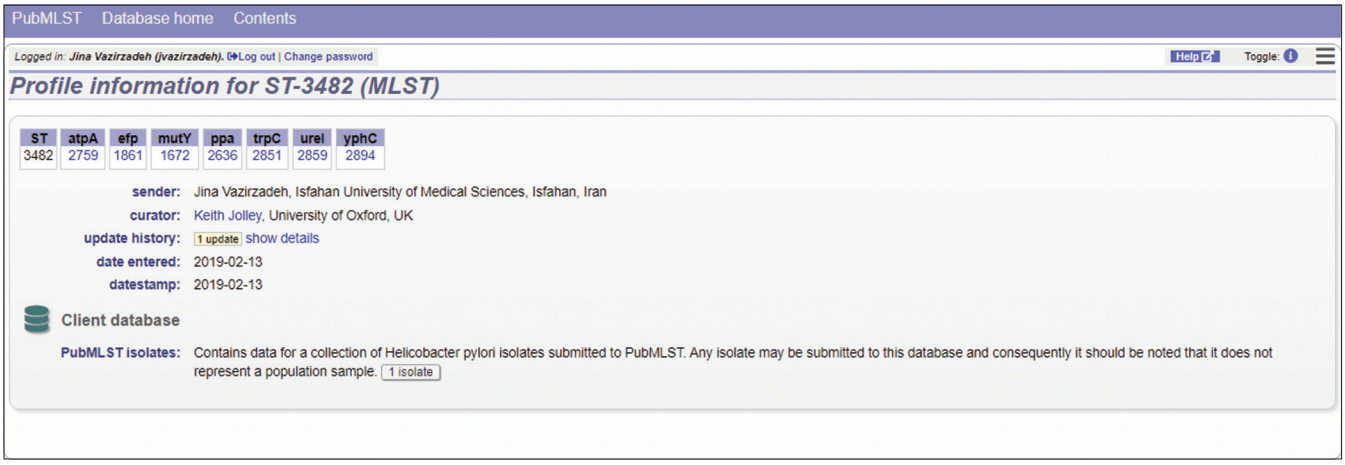

Table 2: Sign ISF1 isolated on Pub multi-locus sequence typing

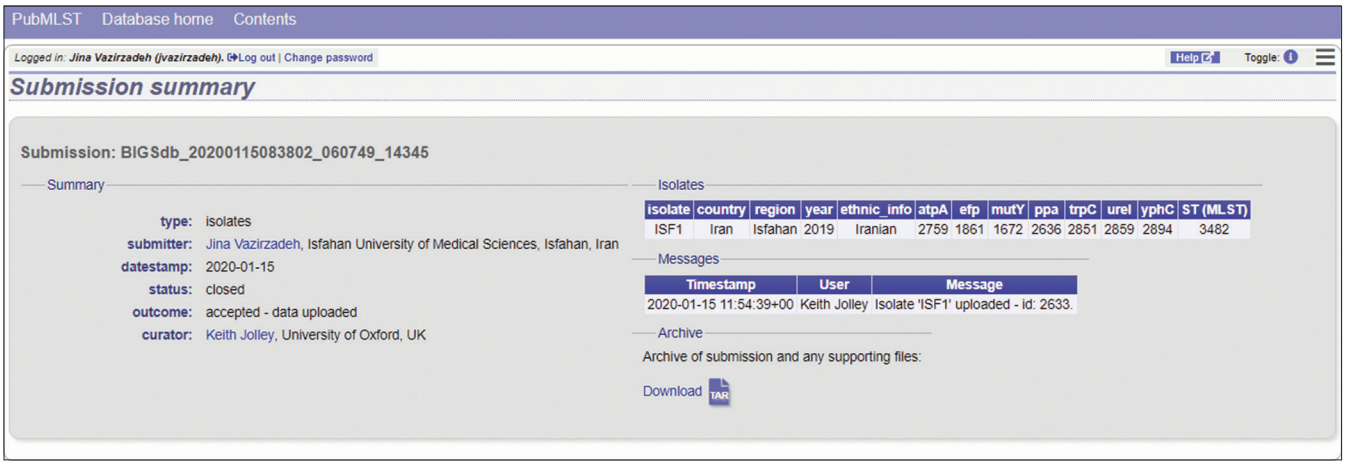

Table 3: Isolated ISF1 information and its id registration in Pub multi-locus sequence typing site