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Original Article

Culturable bacteria in the gastric tissue and diversity of antimicrobial resistance in adults with gastritis

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Abstract

Background: Despite growing knowledge in microbiome studies data about the diversity of cultivable bacteria and their drug resistance patterns in patients with gastritis are scant.

Methods: Two gastric biopsies of 171 symptomatic patients were collected and examined by histological and microbiological methods. Viable bacteria were characterized using conventional techniques, and antimicrobial susceptibility of the isolates was detected.

Results: Acute gastritis, chronic gastritis, and peptic ulcers were detected in 3.5%, 86.5%, and 5.8% of the patients, respectively. Culturable bacteria were isolated from 71.3% of the patients, including *Helicobacter pylori* (*H. pylori)* (26.9%), *Staphylococcus epidermidis* (19.8%), *Micrococcus* (1.1%), *Streptococcus viridans* (*S. viridans*) (13.4%), *Enterococcus faecalis* (*E. faecalis*) (4.6%), *Staphylococcus aureus* (*S. aureus*) (1.7%), and Group D *Streptococcus* (7.1%). Single infection and coexistence of two and three types of bacteria were detected in 43.2%, 15.2%, and 5.2% of the patients, respectively. An odd ratio of 4.4 was measured for *Staphylococcus* spp. in patients with acute gastritis (*P-value* = 0.08). *E*-test results showed intermediate resistance to penicillin in 66.6% of the *S. aureus* isolates, while resistance to vancomycin was detected only in the *S. viridans* (30.4%). Resistance to linezolid was detected in 100%, 17.4%, and 16.7% of *E. faecalis*, *S. viridans*, and group D *Streptococci* isolates, respectively. A high frequency of resistance to penicillin, clindamycin, linezolid, erythromycin, and tetracycline was detected in *S. epidermidis* strains.

Conclusion: Our results highlighted the importance of Gram-positive bacteria in the etiology of gastritis. Resistance of these bacteria to different classes of antibiotics should be considered in the clinical setting.

Keywords: Gastritis, Bacterial infections, Antibiotic resistance, Non-Helicobacter bacteria

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Gastritis is a common multi-factorial disease that is observed in various human populations. It is associated with increased infiltration of neutrophils in the mucosa of the stomach tissue and the formation of histopathological lesions (1). Host genetics, environmental factors, and infections are involved in the disease development. Gastritis has different types, including acute, chronic, and reactive, which develop indirectly as a result of organ dysfunctions or directly through infections by *enteroviruses*, *Epstein-Barr virus*, *Helicobacter* spp., some parasites and fungi, or drugs, alcohol consumption, and immune disorders (2, 3).

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Although this disease can be controlled using appropriate treatment regimens, upon progression, it can lead to cancer (4). Chronic gastritis (CG) is a multistep, aggressive, and lifelong inflammation that starts as a simple (superficial) chronic mononuclear inflammation in two phenotypic forms, atrophic and non-atrophic (5). *H. pylori* is the main cause of non-atrophic gastritis that can progress step by step into atrophic gastritis, which is also linked to the development of intestinal metaplasia, dysplasia, and, ultimately, adenocarcinoma (6). There is enough scientific evidence to consider humans as a superorganism consisting of human and microbial cells (7). While about 50 bacterial phyla are found in the human intestine, the stomach, due to its unfavorable physiological conditions, has been considered a sterile organ for many years (8). Bacterial pathogens, like *H. pylori*, can enter the gastric tissue by transitioning from other organs or growing into the stomach (9). Although the pathogenic nature of *H. pylori* and its cross-talk with the host have been established, the pathogenicity of non-Helicobacter bacteria, or their role as true-colonizers and impact on the maintenance of stomach health, and involvement in the success or failure of therapeutic regimens, remain unclear.

Infection with *H. pylori* and long-term consumption of antacids can promote changes in gastric acidity, secretion of inflammatory cytokines, and pathological events that may support colonization of other microbes (9). Despite the worldwide decrease in infection rate with *H. pylori*, there are warnings about the increasing reports of non-Helicobacter bacteria in the gastric tissue of patients with stomach disorders. Future studies concerning non-*Helicobacter* bacteria are needed to provide evidence indicating the importance and role of these bacteria in the pathogenesis of chronic gastritis, formation of peptic ulcers, and gastric carcinogenesis. A higher level of antibiotic resistance can be expected among the true or transient members of the gastric microbiota in adults, probably through a positive selection pressure following exposure to antibiotics (10). Susceptibility of these bacteria to antimicrobials should be studied to prevent the emergence of resistant variants and related complications in the stomach. Current knowledge about the entity of these bacteria and the status of their susceptibility to antibiotics is limited. In this cross-sectional study, the frequency and diversity of the cultivable bacteria in the gastric biopsy of patients with gastritis, their drug resistance patterns, as well as their link with *H. pylori* infection and histopathological lesions, were investigated.

Methods

Patients and samples: The study was performed on gastric biopsies of patients with gastrointestinal disorders referred to the endoscopic ward of Firoozgar Hospital in Tehran, Iran, from 20 January 2022 to 29 August 2022. Two biopsy samples were taken from the antrum of the stomach of each patient. One biopsy sample was sent to the pathology laboratory of the hospital for histopathological examination, and the other one was transferred to the microbiology laboratory of the Department of Photobiology for culture and phenotypic tests. The study was approved by the Ethics Committee of the Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1400.216, 2021.11.09). An informed consent form was obtained from all the patients and demographic and clinical information of the patients was also recorded in the questionnaire form. The inclusion criteria for the study included all adult patients with gastrointestinal disorders who were subjected to endoscopy. The patients who received antibiotics within the last six weeks underwent stomach surgery, or those with gastrointestinal cancers were excluded from the study.

Histological examination and classification of gastritis: Gastritis was classified by gastroenterologists and pathologists according to severity of the condition, the clinical, endoscopic, or histological findings, and the etiology. Acute gastritis was diagnosed as a sudden inflammation or swelling in the stomach mucosa, which is usually accompanied by abdominal pain, nausea, and vomiting. Chronic gastritis was characterized in cases with long-term gastritis, which causes histological changes and chronic manifestations. In our study, all gastric biopsy samples were histologically examined by hematoxylin-eosin staining in the pathology department, and the type of gastritis was reported based on the clinical and histological parameters as described by the updated Sydney System (11).

Isolation and characterization of *Helicobacter* and *non-Helicobacter bacteria*: For the microbiological analysis, each biopsy specimen was kept in a transport medium consisting of thioglycolate agar (Merck, Germany) and yeast extract (3%, Oxoid, UK). The samples were transferred to the laboratory immediately after collection and homogenized aseptically. A standard method was used for the isolation and characterization of *Helicobacter* spp. A suspension of the homogenized gastric biopsies was inoculated on Brucella blood agar (Merck, Germany) supplemented with antibiotics. The cultures were incubated for 4-7 days in microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2).

The grown tiny colonies with positive urease, oxidase, and catalase reactions were analyzed by microscopy, then purified, and the freshly grown subcultures were conserved at -80 ºC in Brucella broth vials supplemented with bovine serum albumin (50%, v/v). To confirm the identity of *H. pylori* isolates, DNA extraction was done using the alkaline lysis and boiling method described by Ghanbarian et al. (1). Polymerase chain reaction (PCR) was done as follows. A 296 bp fragment of the gene was amplified with the forward (glmM-F, 5'-GGATAAGCTTTTAGGGGTGTTAGGGG-3') and reverse (glmM-R, 5'-GCTTACTTTCTAACACTAACGCGC-3') primers (6). The PCR was done in a 25 µl reaction containing 12.5 µL of 2X master mix (Ampliqon, Denmark), 0.5 µL of each 10 µM primer, 1 µL of DNA (2-5 ng), and 10.5 µL of nuclease-free deionized water. The PCR conditions consisted of initial denaturation at 95 ºC for 5 min, 40 cycles of 94℃ for 30 sec, 61℃ for 30 sec, and 72℃ for 30 sec, and one cycle of final extension at 72 ºC for 10 min. The products were analyzed by gel electrophoresis in 1.5% agarose.

The isolation of Gram-positive and Gram-negative aerobic and facultative anaerobic bacteria was done by the inoculation of 100 µl of the homogenized solution on Blood agar (Merck, Germany) and McConkey agar media (Merck, Germany). Except for *H. pylori*, all the cultures were incubated in aerobic conditions at 37 °C for 24 hours. The grown colonies were characterized based on colony morphology, Gram staining, positive reactions of oxidase and catalase, and biochemical reactions (12).

Antimicrobial susceptibility testing: The disc diffusion method was used to analyze the susceptibility of the *Staphylococcus*, *Enterococcus*, and *Streptococcus* isolates, the most common non-Helicobacter bacteria isolated from patients, to the following antibiotics selected based on the CLSI guideline (Clinical and Laboratory Standards Institute) (13): vancomycin (30 µg), gentamicin (120 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), linezolid (30 µg), cefepime (30 µg), penicillin (10 Unit), clindamycin (2 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg). The minimum inhibitory concentration values of vancomycin and penicillin were determined using the MIC Test Strip (Liofilchem, Italy) according to the manufacturer's instructions. Briefly, a suspension of bacteria with a turbidity of 0.5 McFarland was inoculated onto Mueller–Hinton agar and an antibiotic strip was placed on the center of the agar plate. After 18-24 hours of incubation at 37 ºC, the inhibition zone diameter (IZD) and MIC value of these antibiotics were interpreted and reported according to the standard protocols (13).

Statistical analysis: The statistical analysis was done using IBM Statistics for Windows (Version 22) (SPSS Inc., Chicago, IL, USA). The link between the presences of isolated bacteria in two groups of patients was analyzed by chi-square test. The prevalence and confidence intervals were measured using the normal approximation method where the prevalence was higher than 10%. The exact binomial method was used in cases where the prevalence was less than 10%. A *p*-value less than 0.05 was considered statistically significant.

Results

Study population: In this cross-sectional study, a total of 171 gastric antrum biopsy samples were collected from patients with gastric disorders. The age range of the patients was 17 to 86 years old, which classified as <30 y (9.9%), 30-49 y (45%), and ≥50 y (38%). Out of the total number of patients, 41% of patients (70/171) were males, and 59% of patients (101/171) were females. Acute gastritis, chronic gastritis, and peptic ulcer were detected in 3.5% (6/171), 86.5% (148/171), and 5.8% (10/171) of the patients, respectively.

Prevalence of H. pylori and non-Helicobacter bacteria using culture method: In this study, bacterial colonization in the stomach was detected in 63.7% (109/171) of the patients. *H. pylori* infection (figure 1) and isolation of Gram-positive bacteria (GPB) were confirmed in 26.9% (46/171, 26.9%) and 47.9% (82/171) of the patients. *Staphylococcus epidermidis* (19.8%, 34/171), *Micrococcus* (1.1%, 2/171), *Streptococcus viridans* (13.4%, 23/171), *Enterococcus faecalis* (4.6%, 8/171), *S. aureus* (1.7%, 3/171), and Group D *Streptococcus* (7.0%, 12/171) were among the common GPB isolates from the biopsies in the presence or absence of *H. pylori* infection. Except for *H. pylori*, other Gram-negative bacteria were detected in 9.9% (17/171) of the samples, including *Providencia*, *Citrobacter*, *Klebsiella*, *Escherichia*, and *Pseudomonas* spp. Colonization with single, two, and three types of bacteria was detected in 43.2% (74/171), 15.2% (26/171), and 5.2% (9/171) of the patients, respectively. Details about the co-colonization status of the characterized bacteria are presented in figure 2.

Antibiotic susceptibility of Gram-positive bacteria: Results of the antibiogram showed a high frequency of antimicrobial resistance in the bacterial isolates. *E*-test results showed resistance to penicillin in 66.6% (2/3) of the *S. aureus* isolates, while resistant phenotypes to vancomycin were detected only in the *S. viridans* (43.4%, 10/23) but not in *E. faecalis* and *S. aureus* isolates.

Resistance to linezolid was detected in 100%, 17.4%, and 16.7% of *E. faecalis* (LnzR-EF), *S. viridans*, and group D *Streptococci* isolates, respectively. High frequency of resistance to penicillin (76.5%), clindamycin (88.2%), linezolid (82.4%), erythromycin (91.2%), and tetracycline (88.2%) was detected in *S. epidermidis* isolates. According to *E*-test results, the vancomycin-intermediate resistance phenotype (VIS*A*) was detected in 66.6% (2/3) of the *S. aureus* isolates. None of Enterococci (VR*E*) and 30.4% (7/23) of *S. viridans* (VRSV) isolates showed vancomycin-resistant phenotype according to CLSI and EUCAST standards, respectively. A relatively similar frequency of resistance phenotype was detected for *S. viridans* and group D Streptococci, except for chloramphenicol and erythromycin which was higher among the group D isolates (table 1).

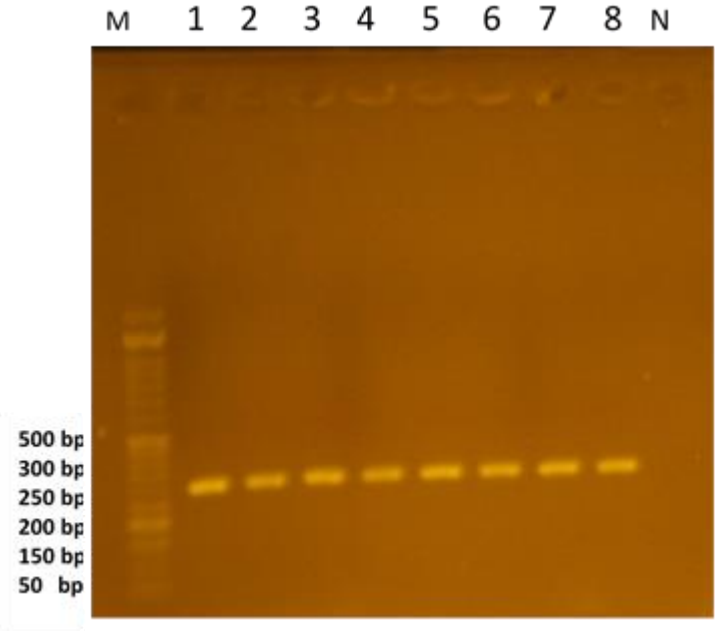

Figure 1. Gel electrophoresis of glmM PCR product for Helicobacter pylori isolates from adult patients with gastritis in Tehran, Iran. Lane 1, DNA marker (50 bp, SMOBIO, South Korea); lanes 1-8, 296 bp PCR products for glmM gene in different H. pylori isolates; N, negative control. Electrophoresis was done in 1.5% agarose gel.

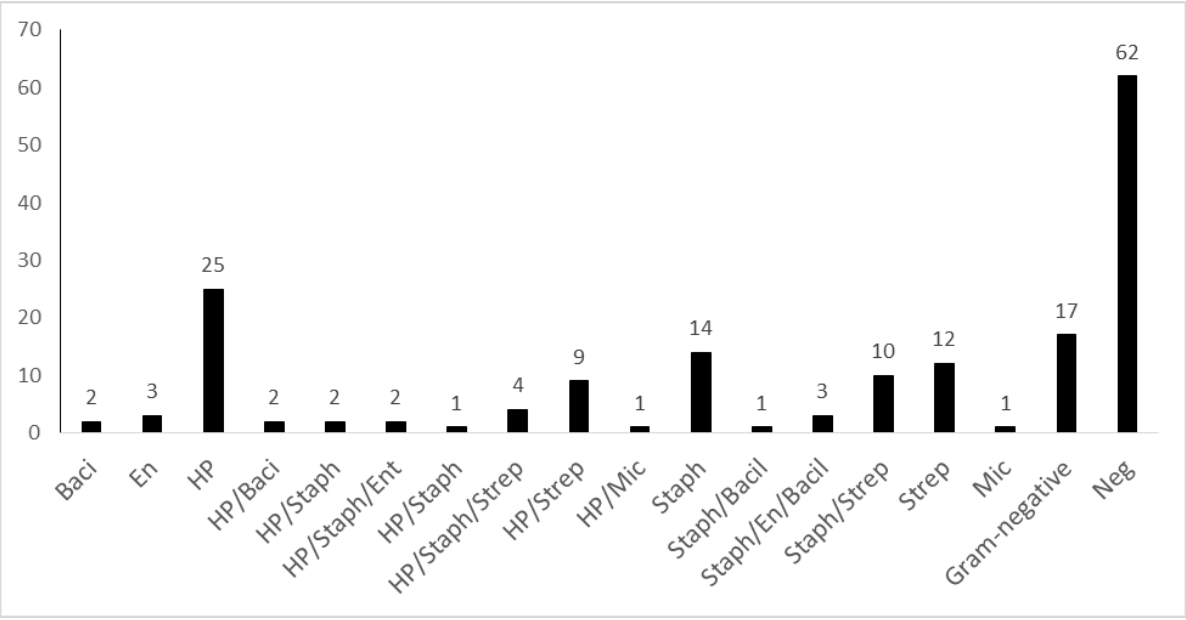

Figure 2. Frequency of bacterial isolates from gastric biopsy samples of patients with gastritis in Tehran, 2020-2023. legend. Abbreviations: HP, *H. pylori*; Staph, *S. aureus* and *S. epidermidis*; Strep, *S. viridans*, and Group D *Streptococcus*; Ent, *E. faecalis*; Mic, *Micrococcus* spp.; Baci, *Bacillus* spp.; Neg, Culture negative.

Table 1. Frequency of antimicrobial resistance patterns among bacterial isolates of the gastric biopsy samples in adult patients in Tehran, Iran

Bacteria	Resistance to antibiotics n, % [a]										
	TET	VAN	CHL	ERY	CEF	LNZ	CP	GEN	PEN	CLN	SXT
Disc diffusion											
***Staphylococcus epidermidis* (n=34)**	30, 88.2%	-	17, 50%	31, 91.2%	-	28, 82.4%	R: 21, 61.8% I: 1, 1.3%	7, 20.6%	26, 76.5%	30, 88.2%	17, 50%
***Streptococcus viridans* (n=23)**	15, 65.2%	-	12, 52.2%	R:10, 43.5% I: 1, 4.4%	10, 43.5%	4, 17.4%	-	-	*E*-test	17, 73.9%	-
***Enterococcus faecalis* (n=8)**	-	-	4, 50%	-	4, 50%	8, 100%	-	0[c]	0	-	-
***Staphylococcus aureus* (n=3)**	0	-	1, 33.3%	2, 66.6%	-	3, 100%	1, 33.3%	2, 66.6%	3, 100%	2, 66.6%	3, 100%
Group D Streptococcus (n=12)	9, 75%	6, 50%	10, 83.3%	9, 75%	R: 6, 50% I: 1, 8.3%	R: 2, 16.7%	-	-	-	-	-
***E*-test**											
***S. aureus* (n=3)**		I: 2, 66.6%							2, 66.6%		
***Streptococcus viridans* (n=23)**		7, 30.4%							0		
***E. faecalis* (n=8)**		0									

Abbreviations: TET, Tetracycline; VAN, Vancomycin; CHL, Chloramphenicol; ERY, Erythromycin; CEF, Cefepime; LNZ, Linezolid; CP, Ciprofloxacin; GEN, Gentamicin; PEN, Penicillin; CLN, Clindamycin; SXT, Trimethoprim/sulfamethoxazole, R: resistant; I, intermediate.

(a) Resistance to different antibiotics was determined using disc diffusion and *E*-test methods.

(b) Interpretation of MIC values for vancomycin and penicillin was done using standard protocols for MIC strips (Liofilchem, Italy) according to CLSI and EUCAST (European Committee on Antimicrobial Susceptibility Testing) protocols (13).

High level gentamicin disc (120 µg) was used for *Enterococcus faecalis* isolates.

Correlation between gastritis and bacterial isolates: Based on the statistical analysis, there was no statistically significant relationship between the presence of *Streptococcus* and *Enterococcus* bacteria and the onset of acute- and chronic gastritis. A possible relationship between *Staphylococcus* infection and acute gastritis was estimated. Accordingly, the odds of developing acute gastritis were estimated 4.4 times higher in the carriers of *Staphylococci* compared with non-carriers (*P*-value= 0.08) (table 2). Similarly, although there was no statistically significant relationship between the presence of these bacteria and chronic gastritis, a higher probability of chronic gastritis was detected in patients with *H. pylori* (Odds of 3.80, *P*-value= 0.06)), and *Streptococcus* spp. infections (Odds of 1.76, *P*-value= 0.39) (table 2). Regardless of diversity, the infection with non-Helicobacter bacteria showed a higher frequency compared with *H. pylori*, both in acute- (4/6, 66.6% *vs.* 0) and chronic gastritis patients (86/148, 58.1% *vs.* 44/148, 29.7%) (table 2). Analyses of the effect of co-colonization of *H. pylori* and non-Helicobacter bacteria with the onset of acute and chronic gastritis did not show a significant difference compared with single *H. pylori* infection or infection status only with non-Helicobacter bacteria (*p*-values of 0.77 and 0.18 for acute and chronic gastritis, respectively) (table 2).

Table 2. The relationship between gastritis and the presence of bacteria in the gastric biopsy samples in adult patients in Tehran, Iran

Gastritis Acute gastritis (n=6)		Positive *n* (%)	Negative *n* (%)	OR*	[95% CI]	*P-value*
***Staphylococcus* spp.**	Negative Positive	3 (50) 3 (50)	132 (81.5) 30 (18.5)	1 4.4	Reference 0.84-22.88	**0.08**
***Streptococcus* spp.**	Negative Positive	4 (66.7) 2 (33.3)	126 (77.8) 36 (22.2)	1 1.75	Reference 0.152-12.73	0.62
Enterococcus faecalis	Negative Positive	6 (100) 0 (0.0)	156 (96.3) 6 (3.7)	1 1	Reference 0-18.71	1.00
H. pyori	Negative Positive	6 (100) 0	116 (71.6) 46 (28.4)	1 0.19	Reference 0.01-3.50	0.14
H. pylori only ***Others only*** ***H. pylori + Others*** ***Negative cultures***		0/6 (0) 4/6 (66.6) 0/6 (0) 2/6 (33.3)	23/162 (14.2) 68/162 (42) 23/162 (14.2) 48/162 (29.6)			0.77
Chronic gastritis (n=148)		**Positive *n* (%)**	**Negative *n* (%)**	**OR***	**[95% CI]**	***P-value***
***Staphylococcus* spp.**	Negative Positive	120 (81.1) 28 (18.9)	15 (75) 5 (25)	1 0.7	Reference 0.22-2.67	0.52
***Streptococcus* spp.**	Negative Positive	113 (76.4) 35 (23.6)	17 (85) 3 (15)	1 1.76	Reference 0.46-9.86	0.39
Enterococcus faecalis	Negative Positive	140 (96.6) 8 (3.4)	19 (95) 1 (5)	1 0.66	Reference 0.07-33.05	0.54
H. pylori	Negative Positive	104 (70.3) 44 (29.7)	18 (90) 2 (10)	1 3.80	Reference 0.85-17.10	0.06
H. pylori only ***Others only*** ***H. pylori + Others*** ***Negative cultures***		22/148 (14.8) 64/148 (43.2) 22/148 (14.8) 40/148 (27.0)	1/20 (5) 8/20 (40) 1/20 (5) 10 (50)			0.18

* Odds ratio, 95% confidence interval and *p*-value obtained using chi-square and Fisher's exact test. Due to low number, data related to duodenal ulcer and gastric ulcer are not presented in this table. Others, the characterized non-Helicobacter bacteria in the absence of *H. pylori* infection.

Discussion

This study aimed to investigate the prevalence of viable and culturable bacteria and the profile of antimicrobial resistance among Gram-positive bacteria in the gastric tissue of adults who suffer from gastritis. The results illuminated the important findings regarding bacterial diversity in the stomach and their influence on disease progression and management. Previous investigations conducted in Iran have demonstrated a wide range of *H. pylori* infection rates, 50.7% (95% CI: 44.4-56.9%) (14). As confirmed in our study, this infection is linked to chronic gastritis (Odds ratio, 3.80). While mono-bacterial colonization showed a higher prevalence in the studied patients, polybacterial colonization was also detected in a high percentage. Increasing evidence supports the co-colonization of Gram-positive and Gram-negative bacteria in the gastric tissue of patients with gastritis (15-17). This study confirmed the presence of bacteria in the stomach

mucosa of 63.7% of symptomatic patients. *Staphylococci*, *H. pylori*, and *Streptococci* were identified as the predominant bacteria in these patients, respectively. Moreover, other Gram-positive bacteria, including *S. epidermidis*, *S. viridans*, and *E. faecalis* were frequently isolated. The presence of these bacteria, particularly in the absence of *H. pylori*, highlights the potential involvement of non-*Helicobacter* bacteria in gastric pathology. No evidence of a significant link between non-*Helicobacter* bacterial infection and gastritis was found. However, the odds ratio of 4.4 for *S. aureus* among patients with acute gastritis, suggest a further investigation into this possible association.

In this study, single colonization with *Staphylococci* was about three times more frequent than co-infection with *H. pylori*. This relationship was supported by other studies (18, 19). The importance of saliva microbiota, like *Porphyromonas* and *Faecalibaculum*, viruses like Epstein-Barr virus, and bacteria, like *S. aureus*, *S. epidermidis*, and *Pseudomonas aeruginosa*, in the onset of non-*Helicobacter* gastritis was illustrated by some studies (18, 20, 21). However, further investigations are required to establish a role for these bacteria in promoting non-*Helicobacter* gastritis. This study showed that *Staphylococci* play a more vital role in the occurrence of acute gastritis than other bacteria. It is challenging to find sufficient data on the microorganisms responsible for acute gastritis. However, some studies have indicated that *Staphylococci* and *Streptococci* may play a role in developing acute gastritis. This is particularly true for immunocompromised patients and individuals with a history of alcohol consumption, smoking, and use of NSAIDs and steroids (22, 23).

None of the isolates in our patients belonged to *S. pyogenes*, which refuses the possible role of its superantigens in the pathophysiology of the disease. Pathogenic Streptococci can adhere and invade the tonsillar epithelial cells and nasal-associated lymphoid tissue by a different mechanism, which may explain their colonization in the gastric tissue and related complications (24). Inconsistent with our findings, the potential contribution of Streptococci in the absence of *H. pylori* in the development of precancerous gastric lesions, like persistent gastritis, atrophy, and intestinal metaplasia, was shown in a longitudinal study (25). More studies are needed to show this relationship. The antimicrobial susceptibility testing results showed the highest frequency of resistance to penicillin and vancomycin in *Staphylococcus* spp. and *S. viridens* isolates, respectively. While the presence of *VRE* and *VRSA* was shown in the stomachs of none of the patients, the high proportion of *VISA*, *VRSV*, and *LnzR-EF* phenotypes supported the pathogenic role of these isolates (26-28). In our study, *S. epidermidis*, *E. faecalis*, *S. viridans*, group D *Streptococci*, and *S. aureus* isolates were more sensitive to gentamicin, linezolid, and vancomycin, respectively. The use of these antibiotics can help patients to cure gastritis. There are currently no guidelines regarding standardized therapy of non-Helicobacter gastritis and bacterial infection of the gastric wall (Phlegmonous gastritis). The administration of broad-spectrum antibiotics was suggested by case studies (29). Our findings suggest that antibiotic resistance among the gastric bacteria could complicate treatment regimens, necessitating the consideration of alternative therapeutic approaches or the development of new antimicrobial agents.

The involvement of resistant pathogens, like multidrug-resistant *Streptococcus* and VR*E,* has been shown in the occurrence of phlegmonous acute gastritis, which highlights the importance of targeted antibiotic therapy in the management of gastric infections (30). Antibiotic susceptibility testing could guide appropriate therapy to minimize the risk of resistance development and treatment failure. Our study emphasizes the requirement for a more comprehensive understanding of the gastric microbiome and its impact on gastritis. Focusing solely on *H. pylori* as the primary pathogen might ignore the impact of other bacteria, especially when considering antibiotic resistance. The potential role of Gram-positive bacteria in the pathogenesis of gastritis and their interaction with host tissues merits further investigation. The detected resistance patterns indicate the importance of routine antimicrobial susceptibility testing in managing effective treatment strategies.

Future directions: Future research should focus on longitudinal studies to track changes in the gastric microbiome over time and their association with disease progression. Additionally, advanced molecular techniques, such as metagenomics, could provide a more comprehensive understanding of the gastric bacterial community and identify potential novel pathogens. Investigating the mechanisms underlying antibiotic resistance in gastrointestinal bacteria could also inform the development of targeted therapies and stewardship programs to mitigate resistant strain spread.

Study limitations: This study may have several limitations, including the small sample size of patients with acute and chronic gastritis, the detection methods of bacteria that may overlook non-culturable or fastidious organisms. This study also lacks longitudinal data, either in patients with gastritis or in a non-gastritis control group, which could provide further insight into the dynamics of bacterial colonization,

the development of resistance in response to antibiotic treatment, and the occurrence or progression of disease. Focusing on Gram-positive bacteria and other microorganisms could give a more comprehensive understanding of the gastric microbial ecosystem. The study found no significant correlation between non-Helicobacter bacterial infections and chronic gastritis, but the clinical significance of these findings requires further exploration. Additionally, the study did not thoroughly examine patients' previous antibiotic usage, which could significantly influence bacteria's presence and resistance patterns in the gastric mucosa. In this study, no samples were collected to check the quality of the disinfection procedure of the endoscopes and related accessories. Although disinfection of endoscopes is routinely done after the sampling procedures and there are local regulations for quality control of the disinfection process of medical devices, random sampling from the endoscope accessories, including biopsy port and channel, air and water channel, is necessary to control cross-contamination during the sampling procedure. In conclusion, this study highlights the diverse and resilient nature of the microbiome in the gastric mucosa of patients with chronic gastritis. The high prevalence of antibiotic resistance among these bacteria highlights a critical challenge in managing gastric infections. While *H. pylori* remains a key pathogen, the potential role of other Gram-positive bacteria in gastric pathology should be considered. Addressing these challenges requires a multifaceted approach, integrating microbiological, clinical, and molecular insights to improve the diagnosis, treatment, and prevention of gastritis and related gastric diseases.

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Ethics approval: This study was performed in accordance with the principles of the Declaration of Helsinki. The Ethics Committee of Tehran University of Medical Sciences approved the study under the code IR.TUMS.SPH.REC.1400.216. (Date: 2021-11-09).

Conflict of interests: The authors have no relevant financial or non-financial interests to disclose.

Authors' contribution: Concept– R.B. and M.A.; Supervision– R.B. and M.A.; Resources – R.B.; Data Collection and/or Processing– M.B. and S.Z.M; Analysis and/or Interpretation – MR.P.; Consultation – M.A. and M.A.; Critical Review – M.A. All authors read and approved the final manuscript.

Consent to participate: Informed consent was obtained from all the participants in the study.

Consent for publication: No personal or clinical details of participants are presented that compromise anonymity. The authors confirm that the participants provided informed consent for the publication of the laboratory results. No additional sampling and medical interventions were done for the patients during the diagnostic procedure according to the hospital regulations.

Data availability: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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