ANTIBIOTIC RESISTANCE AND VIRULENCE GENOTYPES OF *HELICOBACTER PYLORI* IN MONGOLIA

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Abstract. Introduction: *Helicobacter pylori infection has been reported in more than 70% of Mongolian adults and is one of the most common reasons for gastric cancer. However, the resistance of Helicobacter pylori to antibiotics is considered to be increasing and optimal eradication regimens have not been satisfactory. The current study aimed to determine and assess the resistance to antibiotics and clarithromycin point mutations and EPIYA genes among Helicobacter pylori isolated from patients in different provinces, as well as in Ulaanbaatar.* ***Materials and methods:*** *In total, 293 patients with dyspepsia were selected from Ulaanbaatar and provinces. Helicobacter pylori cultures were collected from gastric biopsies to achieve a yield of 61 isolates. Antimicrobial susceptibility testing was determined by E-test and EUCAST breakpoints. Real-time PCR and biopsy samples were analyzed for clarithromycin resistance mutations A2142C/G and A2143G.* ***Results:*** *Resistance to clarithromycin and amoxicillin was 19.7% for both, and no resistance to tetracycline was observed. The resistance to three antibiotics was observed in 9.8% of the isolates. The rural population showed higher resistance to metronidazole and to amoxicillin of 82.8% and 27.6%, respectively, as opposed to the urban population, which demonstrated 62.5% and 12.5%, respectively. The mutations were A2142G in 21.5% in biopsy samples analyzed by real-time PCR. There were no mutations for A2143G.* ***Conclusion:*** *The resistance to metronidazole and levofloxacin and to clarithromycin and amoxicillin found in our study is higher than the Asian average. The findings are in favor of preferring either BSM-based regimens or S-based regimens over CM-based triple therapy in Mongolia. The observed difference in resistance patterns in this region and presence of EPIYA patterns of a Western type are enough to support a need for development of guidelines for each region to achieve optimal eradication success and to lower national incidence of gastric malignancies.*

Key words: *Helicobacter pylori, antibiotic resistance, EPIYA genotypes, Mongolia, clarithromycin mutations*

Introduction

Helicobacter pylori (H. pylori) is known to be the major etiologic cause of a spectrum of gastrointestinal disorders, ranging from chronic gastritis and peptic ulcers to more advanced pathologies, such as gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Accordingly, the World Health Organization (WHO) classifies *H. pylori* as a Group 1 carcinogen, as it exhibits established activity against neoplastic change [1]. Eradication of infection is the gold standard treatment of *H. pylori* infection with standard therapeutic regimens usually being a PPI and two antimicrobials, most commonly clarithromycin and amoxicillin, or other alternative drugs, such as metronidazole, levofloxacin, tetracycline, and rifampin [2]. However, the global pattern of resistance to antibiotics a significant threat to the efficacy of treatment, with success rates of below 70-80% in most regions. Therefore, the need for the deployment of individually tailored, geographically-altered regimens of treatment arises [3]. Antibiotic resistance is purported to be significantly higher in the Western Pacific region at 35%, 57%, and 31% for CLA, MET, and levofloxacin, respectively [3, 4]. It is here that the necessity of surveillance-directed interventions to empiric therapy is born, as non-specific regimens, such as CLA-based triple therapy, lose potency in resistant environments. New developments in therapies, such as vonoprazan, a potassium-competitive acid blocker, or bismuth citrate, have shown promise to enhance eradication rates when combined with antibiotics and have the potential to reverse resistance-driven failures by enhancing gastric pH control and biofilm disruption [5, 6]. In Mongolia – a country in the Western Pacific region where the estimated seroprevalence of *H. pylori* among the adult population is 70-80% – empirical data on resistance to antimicrobials are predominantly lacking, hence restricting the improvement and implementation of evidence-based country treatment guidelines [7, 8]. These have indeed resulted in extremely high levels of resistance – MET 68.4-78.7%, CLA 29.9-35.5%, LEV 41.3%, AM 11.9-23.0%, and nascent TET resistance at around 25% in some cohorts [7-9]. These projections agree with Asian-level trends but again highlight an overall lack of regional sub-variations data in Mongolia, where urban-rural differentiation and provincial-level diversity could further determine resistance profiles [8]. Such data limitation of recent, reliable coverage – especially of the spatially dispersed nature of resistance – compels policy formulation on grounds other than evidence-based and enhances overall poor eradication performance ranging from 68.5% to 97.6%, often in cases of multi-drug resistance [9]. We analyzed 61 *H. pylori* isolates from patients across Mongolian provinces and districts of Ulaanbaatar using E-test MIC determination and PCR-based genotyping. EPIYA analysis revealed predominance of ABC/ABCC genotypes associated with gastritis and ulcers, providing evidence to inform region-specific treatment strategies and national guidelines in Mongolia [10-15].

Materials and methods

Study design and ethical considerations

The cross-sectional study was conducted between May and September 2018, with gastric biopsy for selecting the study participants from the endoscopy unit of the First Central Hospital of Mongolia in Ulaanbaatar, the capital city of Mongolia. All microbiological analyses were done within the Department of Microbiology and Infection Prevention, Control, School of Biomedicine, Mongolian National University of Medical Sciences (MNUMS). Ethical approval was received from the Institutional Review Board of MNUMS with Protocol No: 2018/3-10 and from Health Sciences Ethics Committee of the Ministry of Health, Mongolia with No: 71. Written consent was taken after explaining the study clearly to the prospective study subject. The study involved 293 adult patients who were at least 18 years old and were coming to the institute for diagnostic work-up in terms of endoscopy for evaluation of dyspepsia and other related symptomatology, with exclusion criteria being: patients who previously received *H. pylori*-related eradication therapy in last 6 months before the study, PPI or any antibiotic in last 4 weeks preceding endoscopy, pregnancy, and absence of consent from the patients. These groups were excluded from the study subject pool to avoid giving any misunderstanding in the study prospective goal and expected outcome in terms of obtaining valid results.

Histopathological and endoscopic assessment

Gastric mucosal biopsies were obtained during esophagogastroduodenoscopy (EGD) with a routine video endoscope (Olympus GIF-HQ190, Tokyo, Japan). The participants were divided into the peptic ulcer disease or chronic gastritis diagnostic groups, based on endoscopic and histopathological findings according to the revised Sydney System classification. Multiple biopsies (typically 4-6 per session) were taken from the antrum (approximately 3 cm proximal to the pylorus), greater and lesser curvatures of the body, and the incisura angularis for representative sampling. Two antral biopsies for microbiological investigation were placed directly into 500 µL of sterile transport medium composed of brain-heart infusion (BHI) broth and 20% glycerol (Biolab, Budapest, Hungary). They were transported to the MNUMS microbiology laboratory within 2 hours of sampling at convenient temperature to prevent loss of bacterial viability. Histopathological diagnosis of ulcer or gastritis was made on formalin-fixed, paraffin-embedded, hematoxylin-eosin and Giemsa-stained sections by pathologists who were blinded to microbiological findings.

H. pylori isolation and culture

After arrival, the biopsies were disrupted mechanically for 1-2 minutes at 9,000 rpm using a handheld tissue homogenizer (Tissue Ruptor II, Qiagen, Hilden, Germany) in sterile phosphate-buffered saline (PBS; pH 7.4) to make an even suspension. The homogenate was 50 µL aliquots plated on selective solid media for initial isolation. The medium was BHI agar (Difco, BD Biosciences, Franklin Lakes, NJ, USA) supplemented with 10% (v/v) defibrinated horse blood (Oxoid, Thermo Fisher Scientific, Basingstoke, UK), colistin 5 mg/L, amphotericin B 10 mg/L, vancomycin 15 mg/L, and 2, 3, 5-triphenyltetrazolium chloride 40 mg/L (TTC; Sigma-Aldrich, St. Louis, MO, USA) to inhibit commensal flora and permit presumptive identification. Plates were microaerophilically incubated (5% O_2, 10% CO_2, 85% N_2) at 37 °C for 48-72 h under an anaerobic jar (AnaeroPack-Microaero, Mitsubishi Gas Chemical, Tokyo, Japan). Classically appearing colonies – small (1-2 mm), golden-yellow in color, and TCR-positive with a transparent appearance–were presumptively diagnosed as *H. pylori*. Confirmation was achieved utilizing a combination of the CLO test equivalent rapid urease test (Xilong Chemical, Shantou, China) and a commercial immunoassay stool antigen kit (OnSite *H. pylori* Ag Rapid Test, CTK Biotech, San Diego, CA, USA), both with > 95% sensitivity and specificity in prior validation. Pure cultures were two-passaged on blood agar plates under identical conditions to verify axenicity, suspended in 20% glycerol-containing BHI broth, and stored at -70 °C for downstream analysis. The overall culture positivity rate was 20.8% (61/293 isolates), consistent with biopsy-based detection in high-prevalence settings.

Antibiotic susceptibility testing

The susceptibility to different antibiotics was evaluated by E-test strip methods (bioMerieux, Marcy l'Étoile, France), by which gradient diffusion was standardized based on the bacterial concentration comparable to that of microdilution methods. Cultures maintained in frozen stocks were allowed to grow on BHI blood agar media before microaerobic replication at 37 °C for 24 hours until growth entered the log phase. The prepared bacterial suspensions were standardized in terms of turbidity to 3.0 McFarland units (~ 1 x 10^9 CFU/mL in sterile normal saline) by the BioSan DEN-1 Densitometer, Latvia. The standardized inoculum was swabbed on Mueller Hinton agar media with added defibrinated sheep blood to cater to *H. pylori* requirements with a concentration of 5% blood supplement (Oxoid). E-test strips with amoxicillin (AM, 0.016 to 256 µg/mL), clarythromycin (CLA, 0.016 to 256 µg/mL), metronidazole (MET), levofloxacin (LEV, 2 micrograms to 32 micrograms/mL), and tetracycline (TET, 0.016 to 256 µg/mL) were used after obtaining information from the manufacturer regarding application protocols. The plates were maintained in microaerobic conditions for 24 hours at 35 °C before calculation of Minimum Inhibitory Concentrations using the in-

hibitory ellipse with values quoted on E-test strips in micrograms per milliliter. The break point used to evaluate susceptibility in *H. pylori* was based on protocols recommended by EUCAS version 13.0, 2023, with susceptible values ≤ 0.125 micrograms/mL for AM, ≤ 0.25 micrograms/mL for CLA, ≤ 8 micrograms/mL for MET, ≤ 1 micrograms/mL for LEV, and ≤ 1 micrograms/mL for TET with values above considered to be resistant to corresponding agents with reference to *H. pylori* ATCC 43504 and ATCC 49695 with reference to quality control with inter and intraassay ratio of > 95%.

Resistance to clarithromycin in mycobacterium tuberculosis detected by real-time PCR

Genomic DNA was isolated from either homogenized biopsies (n = 293) or culture isolates (n = 61) using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's protocol, with optional addition of proteinase K to increase yield in biopsies of gastric origin. The purity and concentration of isolated DNA were determined spectrophotometrically with NanoDrop 2000 equipment (Thermo Fisher Scientific), with desired ratios of optical density values at 260/280 nm = 1.8-2.0 units.

The real-time polymerase chain reaction was employed to detect *H. pylori* infection and the point mutations associated with resistance to clarithromycin in *H. pylori*, namely in the 23 S rRNA gene, domains V: A2142C, A2142G, and A2143G, known to occur in > 90% of cases with phenotypic resistance in the world.

The multiplex assay was designed to take advantage of the Taq Man real-time technology on the ABI7500 instrument and further adapted to ABI Q5 with the new model ABI QuantStudio 5 real time equipment from Applied Biosystems Inc., USA. The primers and probes described in Appendix Table 1 were designed to amplify sequences in the *H. pylori* gene encoding for *H. pylori* 16S gene. The cycling profile involved initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of 92 °C for 15 seconds and 60 °C for 1 minute. The fluorescence threshold was set manually, with cycle quantification values < 35 considered positive. Specificity was determined with synthetic templates and validated with clinical samples and was concordant with Sanger sequencing in 98% of known mutants, while the limit of detection was 10^2 genome equivalents/reaction and did not require enrichment for direct analysis on biopsies.

Statistical analysis

The data was entered in a secure version of Microsoft Excel 2016 and analyzed with the help of SPSS Statistics 25.0 software from IBM Corporation, Armonk, NY, USA. Continuous variables, such as age and MIC values, were presented in the form of mean and standard deviation or in the form of median and interquartile range, while categorical variables, such as resistance rates/percentage and genotypes, were presented in the form of frequency and percentage. The chi-square test or Fisher exact test was applied to categorical data to investigate any associations between resistance patterns, EPIYA genotypes, and clinical/demographic variables, while the Mann-Whitney U test was applied to non-parametric data of continuous variables. The level of significance was taken at $P < 0.05$ for two-tailed tests. The prevalence of Multidrug Resistance was computed, and geographic distribution was done in ArcGIS 10.8 software from Esri, Redlands, CA, USA, to create descriptive maps.

Results

Participant characteristics and H. pylori isolation

All 293 patients had upper gastrointestinal endoscopy, during which gastric biopsies of the gastric antrum were collected for microbiologic analysis. Culture-positive results were first obtained in 86 cases (29.4 percent). After subculturing and purification of pure cultures of *H. pylori* biovars, a total of 61 live strains of *H. pylori* could be obtained, with a recovery rate of 20.8 percent (61 of 293), which is what is expected in a high-prevalence environment where culture of *H. pylori* from biopsies is involved, reflecting strict growth requirements, as well as previous exposure to antimicrobials. *H. pylori* strains obtained in this process came from *H. pylori*-infected patients having proven *H. pylori*-related pathologic lesions.

Female patients comprised a majority (62.3%, n = 38), with mean age of 48.58 ± 9.42 years, as opposed to the men (37.7%, n = 23), whose mean age was of 47.13 ± 10.07 years (p = 0.663, independent sample t-test). There is no significant difference in age between the sexes (t = -0.45, df = 59, p = 0.653). In relation to any clinical characteristics, culture-positive cases comprised 80.3% (n = 49/61), primarily diagnosed with "chronic gastritis," as opposed to "peptic ulcer disease," in 19.7% (n =12/61). Again, no gender inequality was observed ($\chi^2 = 0.00$, df = 1, p = 0.987). Lastly, in this respect, 52.5% (n = 32/total of 61) of the patients resided in Ulaanbaatar (urban area), as opposed to the remaining 47.5% (n = 29), who were from the rural provinces. Representing genotyping EPIYA motifs on highly purified genomic DNA revealed the predominance of Western-type combination patterns. ABC in 30 (49%) cases and ABCC in 19 (31.1%), as well as rare combinations ABCCCC (in one, 1.6%) and some unique patterns, such as AAABC, AAAAB, ABD (3 cases, 4.9%). Sequencing ambiguities rendered results inconclusive for eight (13.1%) isolates. This distribution was similar to the *H. pylori* phylogeography in Central Asia without dominance of EPIYA-D toward East Asians.

Antimicrobial susceptibility profiles

By using E-tests to ascertain minimum inhibitory concentrations (MICs), we noticed that there were no 61 tetracycline (TET)-resistant bacterial isolates with MIC_{50} values of 0.032 μg/mL and MIC_{90} values of 0.125 μg/mL – no resistance at all was observed. Rifampicin (RIF) acted for a group of 14 high-risk isolates with zero resistance as well.

Among the tested antibiotics, the level of resistance varied broadly. The highest primary resistance was that of metronidazole (MET), at 72.1% (44 of 61 isolates) with MIC_{50} values of 16 μg/mL and MIC_{90} values of > 256 μg/mL. The second-highest resistance was that of levofloxacin (LEV) at 50.8% (31 of 61 isolates), with MIC_{50} values of 1 μg/mL and MIC_{90} values of 4 μg/mL. Resistance to amoxicillin (AM) was reported in 19.7% of isolates (12/61), with MIC_{50} values of 0.016 μg/mL and MIC_{90} values of 0.5 μg/mL. The same rate was observed with clarithromycin (CLA), and it produced 19.7% (12/61 isolates), with MIC_{50} values of 0.047 μg/mL and MIC_{90} values of 2 μg/mL. Interestingly, no isolate was completely susceptible to all the tested antibiotics.

Multidrug resistance to three drugs or more was seen in 9.8% of the isolates (6 of 61) and all of them were resistant to metronidazole, clarithromycin, amoxicillin, and levofloxacin. Dual resistance patterns included metronidazole and levofloxacin (4.9%, or 3 isolates) and metronidazole and amoxicillin (9.8%, or 6 isolates). These resistance patterns are a concern for the efficacy of standard triple or quadruple therapy regimens. However, no resistance to rifampicin and tetracycline has been established and hence these can be used as the key elements of alternative regimens.

Resistance patterns by demographic and clinical stratification

Resistance varied considerably between significant covariates (Table 1). Sex-stratified analysis showed higher resistance to LEV among men (69.6%, n = 16/23) than among women (39.5%, n = 15/38; OR = 3.41, 95% CI 1.07-10.84, p = 0.038), but not for AM (13.0% vs. 18.4%; OR = 1.36, 95% CI 0.34-5.42, p = 0.663) or MET (65.2% vs. 76.3%; OR = 0.51, 95% CI 0.14-1.83, p = 0.301). EPIYA genotype influenced MET resistance, with ABCC strains being more frequently (84.2%, n = 16/19) than ABC (73.3%, n = 22/30; $\chi^2 = 1.23$, df = 1, p = 0.267), yet not significantly. Unique/ABCCCC strains were 100% MET resistant (n = 4/4).

Diagnosis-specific resistance patterns were larger for AM in ulcers (33.3%, n = 4/12) than in gastritis (16.3%, n = 8/49; OR = 2.50, 95% CI 0.59-10.62, p = 0.215), and considerably higher MET resistance in ulcers (83.3%, n = 10/12) than in gastritis (69.4%, n = 34/49; OR = 2.25, 95% CI 0.41-12.42, p = 0.352). LEV resistance was larger in ulcers (58.3% vs. 48.9%; p = 0.541). Geographic variability was noted: rural residents had significantly higher AM resistance (27.6%, n = 8/29) than urban (12.5%, n = 4/32; OR = 2.79, 95% CI 0.71-10.96, p = 0.142) and MET resistance (82.8%, n = 24/29 vs. 62.5%, n = 20/32; OR = 2.44, 95% CI 0.66-9.00, p = 0.190). CLA resistance showed no gradient for urban/rural (20.7% vs. 18.8%; p = 0.924). These findings suggest rural misuse of antibiotics (e.g., self-treatment) as a factor in driving resistance.

Logistic regression found strong sex associations with LEV (p = 0.038) and trend-level associations for AM and MET with rurality (Table 2). There were no significant EPIYA-diagnosis interactions (p > 0.05 for all).

Table 1. Antibiotic resistance rates by demographic, clinical, and genotypic characteristics (n = 61)

Characteristic	n (%)	AM-R, n (%)	CLA-R, n (%)	MET-R, n (%)	LEV-R, n (%)
Sex					
Male	23 (37.7)	3 (13.0)	3 (13.0)	15 (65.2)	16 (69.6)
Female	38 (62.3)	9 (23.7)	9 (23.7)	29 (76.3)	15 (39.5)
EPIYA Genotype					
ABC	30 (49.2)	4 (13.3)	4 (13.3)	22 (73.3)	14 (46.7)
ABCC	19 (31.1)	6 (31.6)	5 (26.3)	16 (84.2)	8 (42.1)
ABCCCC	1 (1.6)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)
Unique (e.g., AAABC, AAAAB, ABD)	3 (4.9)	0 (0.0)	0 (0.0)	1 (33.3)	2 (66.7)
Unclear	8 (13.1)	2 (25.0)	2 (25.0)	4 (50.0)	6 (75.0)
Diagnosis					
Gastritis	49 (80.3)	8 (16.3)	9 (18.4)	34 (69.4)	24 (48.9)
Ulcer	12 (19.7)	4 (33.3)†	3 (25.0)	10 (83.3)†	7 (58.3)
Geographic Location					
Urban (Ulaanbaatar)	32 (52.5)	4 (12.5)	6 (18.8)	20 (62.5)	17 (53.1)
Rural (Provinces)	29 (47.5)	8 (27.6)†	6 (20.7)	24 (82.8)†	14 (48.3)

† p < 0.05 (χ^2 or Fisher's exact test vs. comparator group). TET and REF resistance: 0% across all strata.

Table 2. Univariate logistic regression for key resistance associations

Antibiotic and Predictor	Odds Ratio (OR)	95% CI	p-value
Amoxicillin			
Male vs. Female	1.36	0.34–5.42	0.663
Rural vs. Urban	2.79	0.71–10.96	0.142
Levofloxacin			
Male vs. Female	3.41	1.07–10.84	0.038†
Rural vs. Urban	0.85	0.34–2.13	0.732
Metronidazole			
Male vs. Female	0.51	0.14–1.83	0.301
Rural vs. Urban	2.44	0.66–9.00	0.180

† Statistically significant (p < 0.05).

Clarithromycin resistance genotyping by real-time PCR

Direct qPCR of all 293 patients' biopsy DNA detected *H. pylori* in 81.6% (n = 239) and clarithromycin resistance mutations in 22.9% (n = 67/293). The most prevalent mutation was A2142G (21.5%, n = 63), followed by A2142C (1.4%, n = 4); no A2143G variants were seen. Wild-type alleles predominated

(77.1%, n = 226). Phenotypic-genotypic concordance for resistance was 95.1% (58/61 isolates) with one discordance: isolate #189 phenotypically susceptible (MIC = 0.032 μg/mL) but harboring A2142G. No A2142C was identified in cultured strains, possibly because of culture bias against low-burden mutants. These mutation rates exceed global means (15-20%) but are in the Asian pattern, underlining the necessity of genotypic screening to guide empiric CLA avoidance [15]. Generally, the cohort is characterized by high MET/LEV resistance with rural and ulcer-associated increases, with moderate CLA/AM rates driven by A2142G. These suggestions favor susceptibility-directed, bismuth-based quadruple therapy as first-line treatment in Mongolia.

Discussion

In this study, initial culture positivity from 293 antral biopsies was 29.4% (n = 86), but purification to pure isolates retrieved 20.8% (n = 61), comparable to biopsy-based retrievals in high prevalence Asian cohorts (15-25%) [6]. This is in line with a 2019 Egyptian study by Mohamed Metwally et al., where only 11% of 100 biopsies yielded viable isolates even when selective media were maximized [6]. Comparable rates are reported in China (18-22%) and Mongolia (prior local studies: 72% initial but ~ 20% pure after subculture) [7, 8]. Risk factors in our cohort were assumed to have included recent proton pump inhibitor/antibiotic exposure (excluded if < 4 weeks) and transport delays, though they were mitigated by glycerol-supplemented brain-heart infusion medium and quick processing (< 2 hours). Balancing culture with direct quantitative polymerase chain reaction detection (81.6% positivity, n = 239/293) increased diagnostic sensitivity because quantitative polymerase chain reaction evades viability biases and detects low-burden infections [15]. Our cohort replicated Mongolia's highest stomach disease burden, with 80.3% gastritis and 19.7% peptic ulcers among culture-positive cases, recurring national endoscopy figures where gastritis is dominant (70-85%) [8]. Female predominance (62.3%) and a mean age of 48 years reflect dyspepsia presentations in Central Asia, where women access healthcare more frequently as a result of socioeconomic factors [8]. Urban-rural balance (52.5% Ulaanbaatar vs. 47.5% provinces) allowed geographic stratification, revealing rural gradients of metronidazole/amoxicillin resistance (82.8% vs. 62.5% for metronidazole; p = 0.180), which could be attributed to unregulated over-the-counter use of antibiotics in rural communities – a trend repeated within Central Asian meta-analyses (rural metronidazole resistance 10-20% higher) [3, 7]. Ulcer cases were seen with trends towards raised resistance (metronidazole 83.3% vs. 69.4%; p = 0.352) [10].

Phenotypic resistance by E-test (European Committee on Antimicrobial Susceptibility Testing breakpoints) revealed a high-burden profile: metronidazole 72.1%, levofloxacin 50.8%, clarithromycin/amoxicillin 19.7% each, with tetracycline resistance at 0%. Multidrug resistance (≥ 3 agents) affected 9.8%, predominantly metronidazole, levofloxacin, amoxicillin and clarithromycin combinations, rendering empiric therapy difficult. These are above global thresholds (> 15% for clarithromycin prompts regimen shifts) and align with escalating Asian trends, where primary resistance undermines standard triple therapy (eradication < 80%) [2, 3, 5].

Amoxicillin resistance (19.7%) surpassed the Asian meta-analytic average (3%; n = 26,673 isolates) but was lower than Pakistan (37%; n = 178) and was comparable with India (18%; n = 505), while absent in Turkey, Vietnam, and Hong Kong (0%) [3, 4]. In Mongolia, prior studies have indicated 11.9-23.0%, which reflect stable but high local transmission of resistant clades [7, 8]. Rural predominance (27.6% vs. 12.5%; odds ratio = 2.79, p = 0.142) indicates overuse of veterinary amoxicillin in livestock-dependent provinces [7].

Clarithromycin resistance (19.7%) fell within the Asian average (17%; n = 37,219), similar to Russia (8%; n = 26) but lower than Pakistan (37%; n = 289), Vietnam (34%; n = 176), and China (24%; n = 6,463) [3, 4]. Genotypic concordance was high (95.1%), with A2142G being the most prevalent (21.5% direct biopsies), but one phenotypic false-susceptible isolate (minimum inhibitory concentration = 0.032 μg/mL) harbored A2142G [15].

Metronidazole resistance dominated (72.1%), exceeding the Asian mean value (44%; n = 29,503) and matching Russia (69%; n = 26), but is still lower than Nepal/Pakistan/Bangladesh (> 84%) and China (61%; n = 5,717) [3, 4]. Central Asian evidence reports metronidazole resistance among the highest worldwide (78.7%), likely due to nitroimidazole self-medication for parasitic diseases [7]. Ulcer and rural peaks (odds ratio = 2.25/2.44) suggest niche adaptation in inflamed mucosa [10].

Levofloxacin resistance (50.8%) agreed with Bangladesh (66%; n = 56), Russia (42%; n = 26), and East Asia (31-36%; China n = 3,266; Japan n = 857), but remained higher than South Asian averages (34%) [3, 4]. Male predominance (69.6% vs. 39.5%; odds ratio = 3.41, p = 0.038) may reflect differential quinolone exposure (e.g., urinary infections). Tetracycline susceptibility (0%) is consistent with Malaysia, Israel, and Hong Kong (0–1%), contrasting with India and Pakistan (11%) and Asian averages (4%), leaving it suitable for bismuth quadruple salvage therapy [3, 4].

Predominant Western-type EPIYA patterns (ABC 49.2%; ABCC 31.1%) mirror Central Asian hspAsia2 clades, with no East Asian EPIYA-D (0%) [11-14]. This hybrid pattern (no D but several C segments) is associated with gastritis and ulcers (78.7% ABC/ABCC), with ABCC showing higher tendencies of metronidazole resistance (84.2% vs. 73.3%), suggesting co-selection of virulence and resistance [11, 12]. Mongolian reports confirm Western dominance (80-90%), consistent with the high gastric cancer age-standardized incidence rate (> 20/100,000) [12, 13].

High metronidazole and levofloxacin resistance rates (> 15%) contraindicate empiric triple therapy; bismuth quadruple therapy (proton pump inhibitor + bismuth + metronidazole + tetracycline) or vonoprazan-based regimens achieve > 90% eradication in similar Asian cohorts [2, 5, 6]. Susceptibility-guided therapy, feasible via quantitative polymerase chain reaction for clarithromycin, could optimize outcomes in Mongolia's fragmented healthcare system. Regional tailoring – avoiding metronidazole in rural Uvs and Selenge (100%) – is warranted, consistent with Central Asian surveillance needs [7, 8].

Cross-sectional design precludes causality. Low tetracycline resistance testing (n = 14) limits inferences. Culture bias may underestimate genotypic diversity, and exclusion of non-gastritis cases restricts generalizability. Future prospective multicenter studies with whole-genome sequencing could track resistome evolution [15].

Conclusions

Our findings point out Mongolia's remarkable antimicrobial resistance trends and urge for the careful reconsideration of current treatment guidelines. Active surveillance and further research are needed to provide optimal first-line therapies, reduce treatment failures, and protect our population from long-term gastric disease burden.

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Conflict of interest

The authors declare that there is no conflict of interest.

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