Helicobacter pylori, antimicrobial resistance evolution 2012–2020 in Vigo sanitary area, Pontevedra, Spain☆

Helicobacter pylori, *evolución de la resistencia a antimicrobianos 2012–2020 en el área sanitaria de Vigo, Pontevedra, España*

The antimicrobial resistance of Helicobacter pylori *Helicobacter pylori* (*H. pylori*) seriously restricts treatment options[1]. *H. pylori* resistance in Spain to one or more active antimicrobials ranges from 17.9% to 51.2% for clarithromycin (CLH), 32% to 33.3% for rifampicin (RD), 17.8% to 38.7% for levofloxacin (LE) and 27% to 39.2% for metronidazole (MZ), while 27.2%–66.6%; 12.4%–20%; 2.4%–12.8% and 1.4% are resistant to one, two, three and four antibiotics, respectively[2–5]. This study analysed antimicrobial resistance from 2012 to June 2020. Gastric biopsies were cultured in Columbia agar, Chocolate agar and Thayer-Martin agar (bioMérieux and BBL) in microaerophilic conditions for 10 days. The susceptibility study was performed in Mueller Hinton Fastidious Agar (bioMérieux) using the ETEST (bioMérieux). The antimicrobials studied and the breakpoints used were those recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[6]. In total, 49 *H. pylori* isolates were studied, 40 (81.6%) of which were resistant to one or more antimicrobials. Overall, 35% (14/40) of the isolates were resistant to one antimicrobial: 50% (7/14) were resistant to CLH, 35.7% (5/14) to MZ and 14.3% (2/14) to amoxicillin (AMX). No other monoresistances were identified. In total, 65% (26/40) were resistant to more than one antimicrobial. Overall, 69.2% (18/26) were resistant to two antimicrobials. The most common combination was CLH-MZ at 72.2% (13/18), followed by CLH-RD at 11.1% (2/18), CLH-LE at 11.1% (2/18) and LE-MZ at 5.5% (1/18). In total, 23% (6/26) were resistant to three antimicrobials, all of which to the combination CLH-MZ-LE. Overall, 7.6% (2/26) were resistant to four antimicrobials, all of which to the combination CLH-MZ-LE-RD. The evolution of resistance over time shows a spike in the last three years. From 2012 to 2017, out of a total of 18 isolates, 55.5% (10/18) were resistant to at least one antimicrobial. From 2018 to 2020, out of a total of 31 isolates, 96.7% (30/31) were resistant to at least one antimicrobial. This represents an increase of 41.2% ($P = .0003$; χ^2). Table 1 shows the evolution of resistance over time. In terms of combinations of antimicrobial resistance from 2012 to 2017, CLH-MZ accounted for 20% (2/10) and CLH-MZ-LE for 10% (1/10). From 2018 to 2020, the combination CLH-MZ accounted for 36.6% (11/30) and CLH-MZ-LE for 16.6% (5/30), while resistance to CLH-MZ-LE-RD was detected for the first time in 2020 in 6.6% (2/30) of the isolates. In short, the treatment alternatives whose susceptibility data make them the most active against *H. pylori* in this health area are tetracycline (100%), AMX (95.9%) and RD (91.8%). For this research, it was not possible to ascertain whether patients in whom *H. pylori* was isolated had been previously treated, which is a limitation of the study. Despite this, given the high rates of *H. pylori* antimicrobial resistance, we consider it vital to determine antimicrobial susceptibility by gastric biopsy and *H. pylori* culture prior to starting treatment[7].

Increased *H. pylori* antimicrobial resistance could be associated with the use of non-invasive diagnostic techniques such as the breath test and stool antigen tests. The microorganism is not isolated in either case, making it impossible to perform an antimicrobial susceptibility test. As a result, the use of untargeted antimicrobial therapy not determined by an antimicrobial susceptibility test could select resistant strains. To support this theory, variability of antimicrobial resistance has been observed in a single strain of *H. pylori* obtained after culturing gastric biopsies from the gastric antrum and body of a single patient and collected in a single procedure. According to Selgrad et al., this variability in resistance has been identified in 15.2% of patients[8]. All these factors could help to explain the rise in *H. pylori* antimicrobial resistance.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Table 1
Evolution of antimicrobial resistance over time.

	R1[b]	R2[b]	R3[b]	R4[b]	Resistant isolates/total studied
2012–2017 (n[a] = 10)	6/10 (60%)	3/10 (30%)	1/10 (10%)		10/18 (55.5%)
2018–2020 (n[a] = 30)	8/30 (26.6%)	15/30 (50%)	5/30 (16.6%)	2/30 (6.6%)	30/31 (96.7%)

[a] n: number of antimicrobial resistant isolates.
[b] R1: resistance to one antimicrobial; R2: resistance to two antimicrobials; R3: resistance to three antimicrobials; R4: resistance to four antimicrobials.

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Pleural effusion as a manifestation of a Cryptococcal infection in an HIV patient

Derrame pleural como manifestación de una infección criptocócica en un paciente con VIH

A 37-year-old male HIV+ individual (CD4 cell nadir of 153 cells/mm^3 and HIV-1 RNA viral load zenit of 9832 copies/mL), treated with emtricitabine/tenofovir disoproxil fumarate/efavirenz with poor therapeutic adherence and without medical follow up for the last 4 years was admitted to the emergency department with history of 3 days of left sided pleuritic chest pain, mild dyspnea and a temperature of 37.5 °C. He was a daily consumer of amphetamines for 5 years and previously had received treatment for intestinal giardiasis and syphilis. Vital signs at arrival showed BP 110/70 mmHg, temperature 37.5 °C, heart rate 85 bpm, respiratory rate 22, SpO_2 96%. Physical examination revealed decreased breathing sounds over the left lung base. Rest was unremarkable.

The laboratory findings showed a normocytic, normochromic anemia (Hemoglobin 11.7 g/dl) with normal white blood count (WBC) and platelets, a normal renal and liver function and mild increase of C-reactive protein to 1.7 mg/dl. HIV-1 RNA viral load was 122,000 copies/mL and CD4+ T cell count 13 cells/mm^3. Chest X-ray showed a left costophrenic recess blunting and a left perihilar cavitary nodule. A computed tomography (CT) scan was performed revealing moderate left pleural effusion with atelectasis, a thick-walled cavitary nodule in the left superior lobule, and left hilar calcified lymph nodes (Fig. 1A and B).

A thoracentesis was made, with positive lights criteria suggesting exudative effusion [proteins 52 g/L, LDH 173 U/L; serum values: proteins: 63 g/L, LDH 166 U/L, WBC: 3710 cells/mm^3 (49% neutrophils, 37% lymphocytes and 12% mesothelial cells) and adenosine deaminase (ADA) 51 UI/L]. Pleural effusion culture was negative for bacteria. PCR detection for *Mycobacterium tuberculosis* in pleural effusion was negative.

Serum *Cryptococcus neoformans* antigen (CrAg) was tested, detecting titers of 1/64. Treatment with amphotericin B and flucytosine was initiated. A lumbar puncture was practiced ruling out central nervous system infection, allowing treatment simplification to fluconazole in monotherapy. We initiated antiretroviral therapy with bictegravir, emtricitabine and tenofovir alafenamide, and pneumocystis prophylaxis with trimethoprim – sulfamethoxazole.

Finally, *C. neoformans* isolation in pleural effusion culture provided the definitive diagnosis, exhibiting a strain with minimum inhibitory concentration (MIC) 8 μg/ml for fluconazole and 0.06 μg/ml for voriconazole. Based on these results, we switched treatment to voriconazole. The organism was detectable after 82 h of incubation. Subsequently bronchoalveolar lavage was also positive for *C. neoformans* (Fig. 1C).

The patient showed clinical and laboratory improvement, being discharged to complete outpatient treatment with voriconazole and follow-up by the infectious disease department. Eight months after the diagnosis a thoracic CT scan was made, showing mainly a thin-walled cavitated lesion in the apical posterior segment with bronchial communication and resolution of the pleural effusion (Fig. 1D).

Discussion

Cryptococcosis infection is caused mainly by *C. neoformans*, an opportunistic fungal infection that became worldwide relevant when the HIV era started.[1,2] The most common clinical presentations include neurological and pulmonary involvement.[3] Nevertheless, the cryptococcal pleural infection remains rare, with about 50 cases reported in the literature.[4]

In our patient, differential diagnosis based on cavitary pulmonary lesions and lymphocytic pleural effusion was made. Because cryptococcosis may also present an elevated ADA in pleural effusion, it can easily be misdiagnosed as tuberculosis, a far