International Journal of Gastroenterology
2020; 4(2): 63-71
http://www.sciencepublishinggroup.com/j/ijg
doi: 10.11648/j.ijg.20200402.17
ISSN: 2640-1681 (Print); ISSN: 2640-169X (Online)

Evolution of Susceptibilities of *Helicobacter pylori* Strains Circulating in Cameroon to Usual Antibiotics: A Three-year Study

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To cite this article:
Eyoum Bille Bertrant, Tali Nguefak Lionel Danny, Fotso Chimi Serge, Malongue Agnes, Ateba Mbassa Roger, Kuiate Jules Roger, Kouitcheu Mabeku Laure Brigitte. Evolution of Susceptibilities of *Helicobacter pylori* Strains Circulating in Cameroon to Usual Antibiotics: A Three-year Study. *International Journal of Gastroenterology.* Vol. 4, No. 2, 2020, pp. 63-71. doi: 10.11648/j.ijg.20200402.17

Received: October 9, 2020; **Accepted**: October 22, 2020; **Published**: November 19, 2020

Abstract: This study was conducted to estimate the evolution of antimicrobial susceptibilities of *H. pylori* strains isolated in Cameroon from 2014 to 2016 in relation to sociodemographic factors and clinical outcomes. A total of 278 *H. pylori* strains were isolated from patients with gastroduodenal disorders and tested for their susceptibility to nine antibiotics using the disc diffusion method. With time, a significant decreased of resistance was observed against clarithromycin (31.11 to 3.20%, $p<0.0001$), erythromycin (66.69 to 9.6%, $p<0.0001$) and metronidazole (86.67 to 69.6%, $p<0.0001$). Resistance to tetracycline (0 to 2.4%) and doxycycline (0 to 1.6%) slightly increase with time. Resistance to amoxicillin (95.5 to 94.4%), ciprofloxacin (4.44 to 4%) and levofloxacin (0 to 0.8%) was relatively constant. No resistance was detected against rifampicin with the time. A significant decrease of resistance was detected against metronidazole / clarithromycin (22.22 to 1.6%, $p<0.0001$) and amoxicillin / clarithromycin (26.67 to 3.2%, $p<0.0001$). Among the tested isolates, 0.92 and 2.4% elicited triple resistance to metronidazole / tetracycline / amoxicillin in 2015 and 2016, and 0.8% quadruple resistance to metronidazole / tetracycline / clarithromycin / amoxicillin in 2016. Higher resistance rate was noticed as age increase and among female compared to men. Our data showed evolution in the antimicrobial susceptibilities of *H pylori* strains circulating in our milieu with time. This finding highlights the need of monitoring periodically *H. pylori* resistance profile to antibiotics in order to determine the adapted treatment for this infection.

Keywords: *Helicobacter pylori*, Antibiotics, Resistance Evolution, Cameroon

1. Introduction

Peptic ulcer disease is a major public health problem because of its high prevalence, its complications and its cost. Since the discovery of *Helicobacter pylori by* Warren and Marshall in 1982 [1], peptic ulcer disease is mostly curable with a week's course of antibiotics and antisecretory drugs [2, 3]. Regimen used in *H. pylori* eradication are triple and quadruple therapy [4, 5]. Triple-therapy regimen consists of proton pump inhibitor and two antibiotics: amoxicillin and clarithromycin, or metronidazole and clarithromycin while quadruple therapy consists of proton pump inhibitor, bismuth and two antibiotics: amoxicillin plus clarithromycin, or metronidazole plus tetracycline [4, 5]. Other treatments have also been proposed, including metronidazole, as well as tetracycline, fluoroquinolones, and rifamycins [4, 5]. However, these treatments, mainly because of resistance have become less effective. A recent study conducted

throughout Europe shown that *H. pylori* resistance rates among adults were 17.5% for clarithromycin and 34.9% for metronidazole, and were significantly higher for clarithromycin and levofloxacin in Western/Central and Southern Europe (>20%) than those in Northern European countries (<10%) [6]. On the other hand, *H. pylori* resistance pattern within a particular population may varies with time. Resistance to metronidazole has increased from 26.7 to 47.2% in 2010 and 2015 respectively, while tetracycline resistance increased from 5.9 to 11.7% in the same period (7, 8). In the same way, resistance to clarithromycin has been found to increase from 17.2% in 2010 to more than 19.7% in 2017 [7-9].

In Africa, resistance rate to MET of 90% was found by Seck in Senegal [10], 100% by Sherif et al. in Egypt [11], 56% by Chaabouni in Tunisia [12]. Against macrolides, resistance rate of 4 and 17.5% were found respectively in Egypt [11] and in Tunisia [12]. In Cameroon, resistance rate of 97.85% was found against MET [13]; 97.14 and 85.6% against amoxicillin respectively in 2018 and 2006; 13.57 and 44.7 against clarithromycin respectively in 2018 and 2006 [13, 14]. Taken into account this widespread emergency of MDR *H. pylori* strains, the Maastricht consensus meeting IV recommended a regular and periodical investigation of *H. pylori* resistance profile to the most commonly used antibacterial medications in different countries [15]. Epidemiological studies has reveal that the prevalence of this infection ranged from 92.2% to 64.34 respectively in 2004 and 2016 [16] in Cameroon. However, there is not data on the timely progression of *H. pylori* antimicrobial susceptibility in Cameroon. Therefore, the present investigation was aimed at determining the antimicrobial susceptibility progression of *H. pylori* strains isolated from 2014 to 2016 to nine routinely used antimicrobial agents in order to adopt an antibiotic regional Programs. The relationship between the resistance of *H. pylori* isolates with age, sex and endoscopic clinical outcomes was also examined.

2. Methods

2.1. Sample Collection

H. pylori isolates were recovered from gastric biopsies of patients with gastro-duodenal disorders undergoing endoscopy at Laquintinie Hospital and General Hospital both in Douala, Cameroon from August-2014 to December-2016. None of the patients had received neither antimicrobial therapy nor proton pump inhibitors for at least 2 weeks prior to endoscopy. The specimens that tested positive for urease test were placed in the sterile tubes containing 3-4 ml sterile normal saline for isolation within 4 h.

2.2. Isolation and Identification of H. Pylori

The biopsies were ground and about two drops of homogenates were inoculated into supplemented Columbia agar (Columbia agar + 5% (v/v) lacked horse blood and 1% (v/v) Vitox) for 48 to 72 h under microaerophilic conditions. The colonies gotten from this were identified using Gram staining and urease, oxidase, catalase reactions. Isolates that exhibited Gram negative curved rods and were positive for catalase, oxidase and urease tests were considered as *H. pylori*. The isolates were suspended in Eppendorf tubes containing Brain Heart Infusion broth supplemented with 5% horse serum (BHI-serum) with 30% glycerol and stored at -80°C until used.

2.3. Antibiotic Susceptibility Test

A total of 278 *H. pylori* isolates were recovered and submitted to the antibiotic susceptibility test using Kirby Bauer disk diffusion method (CLSI, 2015) [17]. The following antibiotic disks were tested: metronidazole (50µg, Bioanalyse), clarithromycin (15 µg, Bioanalyse), erythromycin (15 µg, Bioanalyse), amoxicillin (30 µg, Himedia), ciprofloxacin (5 µg, Mast diagnostics), levofloxacin (5 µg, Mast diagnostics), tetracycline (30 µg, Himedia), doxycycline (30 µg, Mast diagnostics) and rifampicin (5 µg, Himedia).

All isolates were removed from storage at −80°C and subcultured on supplemented Columbia Agar medium for two passages. 200 µl of inoculum from 48 hour *H. pylori* colonies was prepared at Mac Farlands standard turbidity 3 (6×10^8 CFU/ml) and used to seed supplemented Columbia agar plate. The discs of antibiotics were then placed and pressed on the inoculated agar surface. The place were then incubated at 37°C for 48 hours under microaerophilic conditions. Zones of lysis around the antibiotic discs were measured and the isolates were classified as sensitive or resistant according to the Microbiologist French Society guidelines [18]. The experiment was performed in triplicate and the mean diameters of inhibition zone recorded for each antibiotic was calculated.

2.4. Statistical Analysis

SPSS software, version 13 was used to perform statistical analysis. The significance of antibiotic resistance patterns between groups of patients according to socio demographic factors, clinical outcome was determined using Chi-square and Fisher's exact tests. Difference was considered statistically significant at $p < 0.05$.

3. Results

3.1. Patients

Among the 842 patients enrolled, 616 were *H. pylori* positive. From these positive patients, 278 *H. pylori* clinical isolates was recovered, 152 from women and 126 from men. Their mean age was 43±17 years (range 15 to 60 years). Approximately thirty percent (29.14%) of these subjects were with superficial gastritis, 25.54% with duodenal ulcers, 12.59% with erosive gastritis and 4.32% with gastric cancer. The characteristics of patient are summarized in Table 1.

Table 1. *Distribution of H. pylori isolates according patient's characteristic.*

Variable	Number of isolates (%)
Sex	
Female	152 (54.68)
Male	126 (45.32)
Age	
≤ 20	95 (34.17)
21-30	84 (30.21)
31-40	75 (26.97)
41-50	21 (7.55)
51-60	3 (1.08)
Endoscopic outcome	
Duodenal ulcer	71 (25.53)
Gastric ulcer	37 (13.31)
Bulbar ulcer	9 (3.24)
Superficial gastritis	81 (29.14)
Erosive gastritis	35 (12.59)
Chronic gastritis	5 (1.79)
Gastric cancer	12 (4.31)
Normal endoscopic	25 (8.99)
Others	3 (1.08)
Health facilities	
HLD	159 (57.19)
HGD	119 (42.81)
Overall	278

HLD: Laquintinie Hospital Douala; HGD: General Hospital Douala; Others: Gastric polyp, Hiatus hernia, Ulcer scar, Cardiac incontinence etc.

3.2. Culture and Susceptibility

Out of the 278 *H. pylori* clinical isolates 261 (93.9%) were resistant to amoxicillin, 11 (3.85%) to ciprofloxacin, 3 (2.4%) to levofloxacin, 30 (10.75%) to clarithromycin, 96 (34.5%) to erythromycin, 208 (74.5%) to metronidazole, 4 (1.4%) to tetracycline and 4 (1.4%) to doxycycline. No resistance to rifampicin was detected (Table 2). Dual MET-AMX, MET-CLR, MET-TET, AMX-CLR, AMX-TET, CLR-TET resistance was seen in 204 (73.38%), 17 (6.11%), 4 (1.44%), 26 (9.35%), 4 (1.44%) and 1 (0.36%) isolates respectively. Simultaneous resistance to MET-TET-AMX was detected in 4 (1.44%) cases. Quadruple drugs resistance for MET-TET-CLR-AMX was seen in 1 (0.36%) cases (Table 2).

3.3. Trends in Antibiotic Susceptibility Per Year

In 2014, out of the 45 isolates recovered, 95.56% (43/45) were resistant to amoxicillin, 4.44% (2/45) to ciprofloxacin, 31.11% (14/45) to clarithromycin, 86.67% (39/45) to metronidazole and 66.67% (30/45) to erythromycin (Table 2). Dual resistance to MET-AMX, MET-CLR and AMX-CLR was seen in 38 (84.44%), 10 (22.22%) and 12 (26.67%) isolates respectively (Table 2).

In 2015, resistance rate of 92.59% (100/108) to amoxicillin, 3.70% (4/108) to ciprofloxacin, 11.11% (12/108) to clarithromycin, 75.92% (82/108) to metronidazole, 50% (54/108) to erythromycin, 1.85% (2/108) to levofloxacin, 0.92% (1/108) to tetracycline and 1.85% (2/108) to doxycycline was detected from the 108 clinical isolates recovered (Table 2). Dual resistance to MET-AMX, MET-CLR, MET-TET, AMX-CLR and AMX-TET was seen in 80 (74.07%), 5 (4.63%), 1 (0.92%), 10 (9.26%) and 1 (0.92%) cases respectively. One case (1: 0.92%) of resistance to MET-TET-AMX was detected (Table 2).

Table 2. *Resistance pattern of Helicobacter pylori clinical isolates to antibiotics.*

Antibiotics	Overall resistance (%) n=278.	Resistance in isolates according to year, N (%)			
		2014 n=45	2015 n=108	2016 n=125	P value
AMX	261 (93.9)	43 (95.5)	100 (92.59)	118 (94.4)	0.7443
Cip	11 (3.85)	2 (4.44)	4 (3.70)	5 (4)	0.9906
CLR	30 (10.75)	14 (31.11)	12 (11.11)	4 (3.2)	<0.0001*
LEV	3 (2.4)	0	2 (1.85)	1 (0.8)	/
MET	208 (74.5)	39 (86.67)	82 (75.92)	87 (69.6)	<0.0001*
TET	4 (1.4)	0	1 (0.92)	3 (2.4)	/
ERY	96 (34.5)	30 (66.67)	54 (50)	12 (9.6)	<0.0001*
DOX	4 (1.4)	0	2 (1.85)	2 (1.6)	/
RIF	0 (0)	0	0	0	-
MET-AMX	204 (73.38)	38 (84.44)	80 (74.07)	86 (68.8)	0.1231
MET-CLR	17 (6.11)	10 (22.22)	5 (4.63)	2 (1.6)	<0.0001*
MET-TET	4 (1.44)	0	1 (0.92)	3 (2.4)	/
AMX-CLR	26 (9.35)	12 (26.67)	10 (9.26)	4 (3.2)	<0.0001*
AMX-TET	4 (1.44)	0	1 (0.92)	3 (2.4)	/
CLR-TET	1 (0.36)	0	0	1 (0.8)	/
CLR-MET-LEV	0	0	0	0	/
MET-TET-AMX	4 (1.44)	0	1 (0.92)	3 (2.4)	/
MET-TET-CLR-AMX	1 (0.36)	0	0	1 (0.8)	/

P value comparing data per year, N: number, AMX: amoxicillin, Cip: ciprofloxacin, CLR: clarithromycin, LEV: levofloxacin, MET: metronidazole, TET: tetracycline, ERY: erythromycin, DOX: doxycycline and RIF: rifamycin. *: Significant.

In 2016, from the 125 clinical isolates recovered, 94.4% (118/125) were found to be resistance to amoxicillin, 4% (5/125) to ciprofloxacin, 3.20% (4/125) to clarithromycin, 69.6% (87/125) to metronidazole, 9.6% (12/125) to erythromycin, 0.8% (1/125) to levofloxacin, 2.4% (3/125) to tetracycline and 1.6% (2 / 125) to doxycycline (Table 2). Dual resistance to MET-AMX, MET-CLR, MET-TET, AMX-CLR, AMX-TET and CLR-TET was seen in 86 (68.8%), 2 (1.6%), 3 (2.4%), 4 (3.2%), 3 (2.4%) and 1 (0.8%) isolates respectively. Simultaneous resistance to MET-TET-AMX was seen in 3

(2.4%) cases and quadruple drugs resistance to MET-TET-CLR-AMX in 1 (0.8%) cases (Table 2).

3.4. Overall Evolution of Resistance from 2014-2016

A decreasing resistance rate from 31.11% to 3.20, from 66.69% to 9.6% and from 86.67 to 69.6% was observed against CLR (p<0.0001), ERY (p<0.0001) and metronidazole (p<0.0001). Resistance to TET (0 to 2.4%) and DOX (0 to 1.6%) slightly increase from 2014 to 2016. Resistance to amoxicillin (95.5 to 94.4%), ciprofloxacin (4.44 to 4%) and levofloxacin (0 to 0.8%) was relatively constant with time. No resistance was detected against RIF with the time (Figure 1 and Table 2).

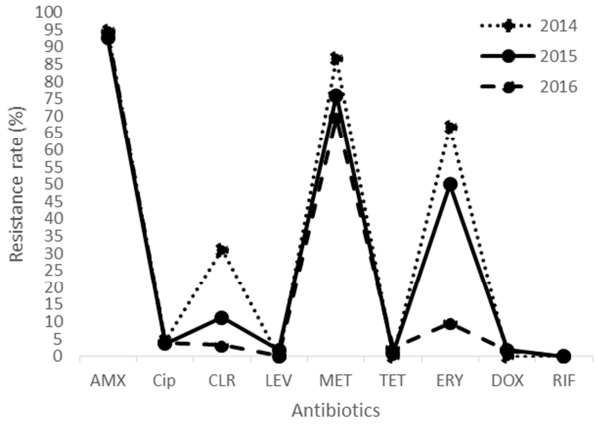

Figure 1. *Resistance of Helicobacter pylori to the tested antibiotics according to year (2014-2016).*

A significant linear decrease of dual resistance was detected against MET-CLR and AMX-CLR with resistance rate decreasing from 22.22 to 1.6% and from 26.67 to 3.2% respectively (p<0.0001 and p<0.0001 respectively) (Table 2 and 3, Figure 1). In 2014, no case of triple or quadruple resistance were detected, whereas in 2015 and 2016, 0.92 and 2.4% cases of resistance to MET-TET-AMX was detected and 0.8% to MET-TET-CLR-AMX in 2016 (Table 2).

3.5. Factors Associated with Antibiotic Resistance

Increasing age was significantly associated with ERY (p=0.038) and DOX (p=0.0001) resistance (Table 4). The resistance frequencies to LEV (p=0.0001), CLR (p=0.013) and ERY (p=0.043) was significantly associated with endoscopic outcome with a high resistance rate among patients with bulbar ulcer and gastric cancer for CLR, gastric ulcer and chronic gastritis for ERY. CLR (p=0.0001), MET (p=0.018) and ERY (p=0.0001) resistance were all significantly higher in subjects enrolled at HLD compared to those from HGD (Table 4).

A significantly higher resistance rate among female compared to men was detected against MET-AMX (p=0.001). Also, significant increase of resistance rate to MET-AMX was noticed as age increase (p=0.026). Again, subjects enrolled at HLD compared to those from HGD were significantly more resistant to AMX-MET (p=0.002) (Table 4).

Table 3. *Resistance rates of H. pylori clinical isolates according to gender, age and clinical outcome.*

Variables	Number of isolates	Antibiotic resistance rate N (%)			
		AMX	CIP	CLR	LEV
Gender					
Female	152	142 (93.4)	8 (5.3)	17 (11.2)	NA
Male	126	119 (94.4)	3 (2.4)	13 (10.3)	3 (2.4)
Age					
≤ 20	95	90 (94.7)	6 (6.3)	7 (7.41)	1 (1.1)
21-30	84	79 (94.0)	4 (4.8)	10 (11.9)	NA
31-40	25	72 (96.0)	1 (1.3)	12 (16.0)	2 (2.7)
41-50	21	17 (81.0)	NA	1 (4.8)	NA
51-60	3	3 (100)	NA	NA	NA
Endoscopic outcome					
Duodenal ulcer	71	69 (97.2)	4 (5.6)	8 (11.3)	NA
Gastric ulcer	37	34 (91.9)	NA	4 (10.8)	NA
Bulbar ulcere	9	9 (100)	NA	4 (44.4)	NA
Superficial gastritis	81	75 (92.6)	4 (4.9)	4 (4.9)	2 (2.5)
Erosive gastritis	35	33 (94.3)	1 (2.9)	3 (8.6)	NA
Chronic gastritis	5	5 (100)	NA	NA	NA
Gastric cancer	12	10 (83.3)	1 (8.3)	4 (33.3)	NA
Normal endoscopic	25	23 (92.0)	1 (4.0)	2 (8.9)	NA
Others	3	3 (100)	NA	1 (23.3)	1 (33.3)
Health facilities					
HLD	159	149 (93.7)	6 (4.2)	26 (16.4)	2 (1.3)
HGD	119	112 (94.1)	5 (3.8)	4 (3.4)	1 (0.8)
Overall	278	261 (93.9)	11 (3.85)	30 (10.75)	3 (2.4)

Table 3. *Continued.*

Variables	Antibiotic resistance rate N (%)				
	MET	**TET**	**ERY**	**DOX**	**RIF**
Gender					
Female	118 (77.6)	3 (2.0)	53 (34.9)	3 (2.0)	NA
Male	90 (71.4)	1 (0.8)	43 (34.1)	1 (0.8)	NA
Age					
≤ 20	72 (78.8)	1 (1.1)	24 (25.3)	NA	NA
21-30	59 (70.2)	3 (3.6)	32 (38.1)	3 (3.6)	NA
31-40	57 (76.0)	NA	29 (38.9)	NA	NA
41-50	17 (81.0)	NA	11 (52.4)	NA	NA
51-60	3 (100)	NA	NA	1 (33.3)	NA
Endoscopic outcome					
Duodenal ulcer	54 (76.1)	NA	28 (39.4)	1 (1.4)	NA
Gastric ulcer	29 (78.4)	1 (2.7)	19 (51.4)	1 (2.7)	NA
Bulbar ulcere	6 (66.7)	NA	4 (44.4)	NA	NA
Superficial gastritis	62 (76.5)	3 (3.7)	22 (27.2)	1 (1.2)	NA
Erosive gastritis	25 (71.4)	NA	11 (31.4)	NA	NA
Chronic gastritis	5 (100)	NA	3 (60)	1 (20.0)	NA
Gastric cancer	8 (66.7)	NA	5 (41.7)	NA	NA
Normal endoscopic	18 (72.0)	NA	3 (12.0)	NA	NA
Others	1 (33.3)	NA	1 (33.3)	NA	NA
Health facilities					
HLD	127 (79.9)	3 (1.9)	87 (54.7)	3 (1.9)	NA
HGD	81 (68.1)	1 (0.8)	9 (7.6)	1 (0.8)	NA
Overall	208 (74.5)	4 (1.4)	96 (34.5)	4 (1.4)	0 (0)

AMX: amoxicillin, Cip: ciprofloxacin, CLR: clarithromycin, LEV: levofloxacin, MET: metronidazole, TET: tetracycline, ERY: erythromycin, DOX: doxycycline, RIF: rifampicin, HLD: Laquintinie Hospital Douala; HGD: General Hospital Douala; Others: Gastric polyp, Hiatus hernia, Ulcer scar, Cardiac incontinence etc, NA: not available.

Table 4. *Factors associated with antibiotic resistance pattern.*

Resistance pattern	P value	Resistance pattern	P value	Resistance pattern	P value
AMX		ERY		AMX-CLR	
Gender	0.462	Gender	0.435	Gender	0.369
Age	0.135	Age	0.164	Age	0.383
Outcome	0.642	Outcome	0.102	Outcome	0.224
Health facilities	0.549	Health facilities	0.436	Health facilities	0.469
CIP		DOX		AMX-TET	
Gender	0.180	Gender	0.499	Gender	0.725
Age	0.280	Age	0.038*	Age	0.294
Outcome	0.691	Outcome	0.043*	Outcome	0.153
Health facilities	0.546	Health facilities	0.0001*	Health facilities	0.726
CLR		MET-AMX		CLR-TET	
Gender	0.728	Gender	0.385	Gender	0.682
Age	0.134	Age	0.0001*	Age	0.282
Outcome	0.013*	Outcome	0.087	Outcome	0.082
Health facilities	0.0001*	Health facilities	0.426	Health facilities	0.304
LEV		MET-CLR		CLR-MET-LEV	
Gender	0.092	Gender	0.001*	Gender	0.222
Age	0.463	Age	0.026*	Age	0.465
Outcome	0.0001*	Outcome	0.116	Outcome	0.211
Health facilities	0.608	Health facilities	0.002	Health facilities	0.367
MET		MET-TET		MET-TET-AMX	
Gender	0.147	Gender	0.353	Gender	0.231
Age	0.526	Age	0.468	Age	0.080
Outcome	0.577	Outcome	0.423	Outcome	0.379
Health facilities	0.018*	Health facilities	0.127	Health facilities	0.275
TET				MET-TET-CLR-AMX	
Gender	0.385			Gender	0.266
Age	0.300			Age	0.374
Outcome	0.535			Outcome	0.557
Health facilities	0.426			Health facilities	0.336

AMX: amoxicillin, Cip: ciprofloxacin, CLR: clarithromycin, LEV: levofloxacin, MET: metronidazole, TET: tetracycline, ERY: erythromycin, DOX: doxycycline, RIF: rifampicin *: Significant.

4. Discussion

The emergence of MDR *H. pylori* strains has become a serious challenge all over the world, as it is the major reason for the low rate of *H. pylori* eradication and the failure of the therapies both in developing [18, 20] and developed countries [21, 22]. This highlights the need for knowledge on the resistance pattern and their timely progression in order to adopt an antibiotic regional Programs. In the present investigation, we evaluated the evolution of antimicrobial susceptibilities of 278 *H. pylori* clinical isolates from Cameroon during a 3-years period (2014 to 2016).

In this study, 93.9% of the tested isolates were resistant to amoxicillin with the resistance rate fluctuation of 95.5 to 94.4% during the study period, indicating a constant high resistance to this drug. This observation is in line with previous studies in Cameroon, showing *H. pylori* high resistance rate of 85.6 and 97.14% respectively in 2006 [14] and 2018 [13]. This high resistance rate to amoxicillin could be the consequence of the wide use of this antibiotic in Cameroon due to its cheapness. Similar reports were noticed in Japan where the authors detected a stable trend of resistance to amoxicillin in their sample isolates with time [23]. However, it is in contrast with some studies revealing an increase trends of primary resistance to amoxicillin in South Korea [24] and a decrease rate from 7.9 to 0% among Bulgarian children in 1996-1999 and 2005–2008 [25].

Resistance rate of 10.75% and 34.5% respectively against clarithromycin and erythromycin was detected among our sample isolates (Table 2). We also found a significant decrease of resistance against CLR ($p<0.0001$) and ERY ($p<0.0001$) during the 3 year study. Similar resistance trend to clarithromycin is observed when putting together previous reports regarding resistance to this antibiotic in Cameroon. In fact, resistance rate to CLR of 44.7% and 13.57 was found respectively in 2006 and 2018 in Cameroon [13, 14]. High cost of clarithromycin limits its use in our milieu [26], this may partially explained such decrease in the resistant rate of the tested isolates to this drug. Our results showed a simultaneous decrease of resistance to both clarithromycin and erythromycin with the time, this may be due to cross-resistance in this class of antimicrobials as previously described [13]. Some data on the timely variability of the susceptibility of this pathogen to macrolides are available in the literature. A stable resistance trend to clarithromycin has been reported in UK and a decrease one in Netherlands, where antibiotic sailing is lower than in others EU countries [27, 28]. Also in Sweden where national antibiotic use is lower, low *H. pylori* resistance rate of 1.0–1.5% to clarithromycin had been noticed [29]. Owing to the growing consumption of macrolides, *H. pylori* resistance has increased in many countries [30]. Increasing macrolide use for pulmonary, ear, nose and throat infections in Japan has been associated with growing clarithromycin resistance from 18.9% to 27.7% respectively between 2002-2003 and 2004-2005 [23]. Similarly, increase resistance rate has been reported against macrolide in Italy (2.1-fold over 15 years) [25], in Bulgaria (1.8-fold over 10 years) [25], in South Korea (4.9-fold over 10 years) [24] and in Japan (1.5-fold over 2 years) [23]. In South Korea, the primary clarithromycin resistance rate increasing from 0 to 13.8% in 1987 and 2003 has been reported [31]. In Bulgaria, the primary clarithromycin resistance increase of 10% from 1996 to 1999 and 18.4% from 2005 to 2008 was observed, probably owing to an increase in the national consumption of macrolides from 0.8 daily doses per 1000 inhabitants in 2004 to 1.8 daily doses per 1000 inhabitants in 2006 [32, 33].

Our findings showed resistance rate of 74.5% against metronidazole and a significant decrease of resistance to the said antibiotic with time ($p<0.0001$). Previous reports in Cameroon regarding resistance to MET did not shown a similar resistance trend to this antibiotic. In fact, resistance rate of 93.2 and 97.85% respectively was revealed in 2006 and 2018 [13, 14], suggesting little or no variation of the resistance against this drug. This high resistance is thought to be linked to the frequency of use of nitroimidazole derivatives in the treatment of digestive and genital parasitic infections [34]. Primary *H. pylori* resistance to metronidazole are reported to be stable in countries such as Bulgaria [24], Japan [23] and The Netherlands [27], unlike in South Korea where an increase resistance rate due to growing use or abuse of metronidazole for parasitic or female genital infections has been reported [31].

Resistance to tetracycline is not widespread. Low rates of 0.3, 1.7 and 2% were detected respectively in Sweden [35], Estonia [36] and Lebanon [37], which are slightly in accordance with the resistance rate of 1.4% detected in this study. Although tetracycline were slightly affected by resistance, low increasing of TET and DOX resistance from 0 to 2.4% and 0 to 1.6% respectively was detected in our milieu. In Cameroon, resistance rate of 43.9 and 2.86% had been reported in 2006 and 2018 [13, 14], suggesting a drastic decrease of resistance to tetracycline from this time elapsed. However, our result as regard resistance rate to TET is similar to that reported in 2018, thus the differences between these resistance trends probably reflect the variation in tetracycline usage between our sample populations. Increasing primary *H. pylori* resistance to tetracycline has been described in South Korea and a stable one in Bulgaria due to decreasing tetracycline consumption from more than 4.2 daily doses per 1000 inhabitants in 1994–1999 to 2.4 daily doses per 1000 inhabitants in 2006 [24, 31].

Although no resistant to fluoroquinolones has been early observed in Cameroon [13], this study showed low resistance rate of 3.85% (11/278) and 2.4% (3/278) respectively against ciprofloxacin and levofloxacin. Our results also showed that resistance to levofloxacin (0 to 0.8%) and ciprofloxacin (4.44 to 4%) was relatively constant from 2014 to 2016, which is seem to be similar to that observed in Bulgaria [24]. However, increasing resistance trend to levofloxacin of 5.1-fold in children and 4.2-fold in adults since 2004 was observed in

Taiwan [38, 39], whereas in South Korea it was ranged from 0 to 33.3% from 1987 to 2003 [31].

Regarding multi-drug resistance pattern, our finding showed that 75% of the isolates were resistant to at least two antibiotics, which is in accordance with the 70% previously reported in Cameroon [13]. This high prevalence of MDR phenotype among *H. pylori* isolates circulating in Cameroon may be attributed to the exhaustive use of antibiotics across the country. Dual resistance to MET-AMX, MET-CLR, MET-TET, AMX-CLR, AMX-TET and CLR-TET was detected among the isolates with a significant decrease against MET-CLR and AMX-CLR. In contrast, increasing double resistance rates to MET-CLR from 2.8 to 3.1% and from 5.0 to 11.2% had been observed at two centers in the UK, and a stable resistance pattern in Bulgaria [40]. Also, Pereira et al. in their study detected two clinical isolates with resistance to MET-CLR and three to metronidazole-clarithromycin-ciprofloxacin simultaneously [41]. Resistance rate range from 0 to 0.92% and to 2.4% was detected against MET-TET-AMX respectively in 2015 and 2016 and 0.8% against MET-TET-CLR-AMX in 2016, indicating an increase of simultaneous resistance with time. Few studies reporting similar low quadruple-drug resistance rate from India (2.5%), Bulgaria (0.7%), Vietnam (1.9%), and Indonesia (2.6%) has been documented [24, 42, 43]. However, these resistance rate were lower than the 15.71 and 5.71% triple and quadruple drugs resistance reported in Cameroon [25] and the 37.9% reported in Chile [42].

Our data also showed a significantly higher resistance rate against AMX-MET among female compared to men (p=0.001). Study conducted in Costa Rica [44] and Mexico [45] also reported similar distribution of resistance to MET according to sex. The widespread use of this antibiotic by women for gynecological infections may explained such observation [34]. Our results also showed a high resistant rate among older compare with younger (p=0.026), may be due to the fact that increase in age enhance the chance of exposure to resistance strains.

5. Conclusion

The present study showed that the resistance patterns of *H. pylori* isolates from Cameroon has changed within the 3 years study. Our data showed significant decrease in resistance of *H pylori* isolates against CLR, ERY and MET, a slightly increase of resistance to TET and DOX, stable state of resistance to amoxicillin, ciprofloxacin and levofloxacin, and a significant decrease of dual resistance against MET-CLR and AMX-CLR from 2014 to 2016 in our milieu. Women and older were the groups showing higher resistance rates to antibiotics. Increasing resistance rates to TET and DOX should be considered as an indicator of abuse and overuse of these antibiotics that ultimately limit their effectiveness for future generations. Even though the resistance to CLR and MET is decreasing, it continues to be worryingly high. Therefore, the frequency of *H. pylori* resistance to current antibiotics should be monitored periodically to determine the adapted treatment for this infection.

Abbreviations

HLD: Laquintinie Hospital Douala, HGD: General Hospital Douala, AMX: amoxicillin, Cip: ciprofloxacin, CLR: clarithromycin, LEV: levofloxacin, MET: metronidazole, TET: tetracycline, ERY: erythromycin, DOX: doxycycline, RIF: rifamycin.

Author Contributions

Kouitcheu Mabeku LB designed the work; Fotso CS, Malongue A and Ateba MR examined the patients, collected patient's clinical data and biopsies sample; Eyoum BB and Tali NLD carried out the analysis; Kouitcheu Mabeku LB and Kuiate JR analyzed the data and wrote the paper.

Declarations

All the authors do not have any possible conflicts of interest.

Acknowledgements

We acknowledge the support of the staffs of the Laquintinie Hospital and General Hospital of Douala-Cameroon who facilitated recruitment of patients for this research. We thank the participating patients for their cooperation.

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