Helicobacter pylori: Prevalence and antibiotic susceptibility among Kenyans

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Background. Helicobacter pylori infection in Kenya is staggeringly high. Evidence links infection of the gastric mucosa by *H. pylori* with subsequent development of gastric pathologies.

Aim. We investigated the prevalence of *H. pylori* in dyspeptic patients, its relationship with gastric pathologies, and associated antibiotic susceptibility profiles, and compared two media to find the appropriate medium that enhances growth and expedites culture and isolation.

Methods. Rapid urease and histological tests were used to screen for *H. pylori*. Culture was performed to test sensitivity and evaluate media. Selective and nutritional supplements were added to culture media (Colombia blood agar and brain-heart infusion agar) for growth enhancement. E-test strips for metronidazole, amoxicillin and clarithromycin were used for susceptibility testing.

Results. The prevalence of *H. pylori* infection in children was 73.3%, and 54.8% in adults. All the *H. pylori* investigated in this study were largely sensitive to clarithromycin (100%, minimum inhibiting concentration (MIC) <2 µg/ml), amoxicillin (100%, MIC <2 µg/ml) and metronidazole (95.4%, MIC <8 µg/ml). There was, however, occasional resistance to metronidazole (4.6%, MIC >8 µg/ml). Both Colombia blood and brain-heart infusion agar, with the supplements, effectively supported *H. pylori* growth. Growth was achieved in an average of 36 hours for primary isolations and 24 hours for subcultures.

Conclusion. The media described here reduce the time required to culture and isolate bacteria and perform susceptibility testing. Despite the high prevalence of *H. pylori* infection, the associated pathology is low and does not parallel *H. pylori* prevalence in the population.

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Increasing evidence links infection of the gastric mucosa by *Helicobacter pylori* with the subsequent development of gastric pathologies. The incidence of *H. pylori* infection in Kenya is staggeringly high. An investigation into the prevalence of *H. pylori* in patients with dyspepsia and asymptomatic controls from the same population, showed high prevalence levels in both groups.[1] Seropositivity for *H. pylori* was found in 98 (71%) symptomatic patients and in 70 (51%) asymptomatic participants. McFarlane *et al*. linked infection of the gastric mucosa by *H. pylori* in Kenya with the subsequent development of gastric atrophy.[2] *H. pylori* prevalence in Kenya shows no difference between HIV-positive and -negative subjects.[3] However, data on *H. pylori* antibiotic sensitivity profiles in Kenya are scarce because of lack of reliable and affordable media on which isolation and susceptibility testing of *H. pylori* can be performed.[4-7] Lack of information on the susceptibility patterns of *H. pylori* strains implicated in infections leads to poor management, resulting in treatment failures. Resistance to metronidazole and clarithromycin has been reported to be highly prevalent and more common elsewhere.[8] It is therefore important to find a *medium* that easily supports the growth of *H. pylori* and allows prompt susceptibility testing. We also investigated the prevalence of *H. pylori* infection in Kenya and associated disease outcomes.

Materials and methods

Patient population

We recruited 696 patients who had presented with dyspepsia at the gastroenterology clinic of the Aga Khan University Hospital, Nairobi, and who had been referred for endoscopy. They were all screened for *H. pylori* by the rapid urease and histological tests. Of the 696 patients, 70 (10%), who had positive stool antigen test prior to endoscopy and who had no history of antibiotic use in the 3 months preceding their visit to the clinic, were selected for *H. pylori* culture. The study was approved by the Aga Khan University Hospital Research and Ethics Committee. Biopsies (2 each from the antrum and corpus) were taken, submerged in 5% brain-heart-infusion agar (BHIA), enriched with 5% fetal bovine serum (FBS) and kept at

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4ºC till processing. All biopsies were processed within 3 hours of extraction.

Culture media preparation

Inactivation of fetal bovine serum

FBS to be used for culture media preparation was completely thawed at room temperature, transferred to a water bath at 56°C and stirred manually every 10 minutes. When the FBS reached 56°C (indirectly measured by the temperature of the water bath), it was incubated for 30 minutes, cooled to room temperature, dispensed in aliquots of 35 ml into tubes labeled 'Inactivated FBS', and stored at -20°C. Sterility control was performed by incubating an aliquot of the FBS at 37°C for 48 hours.

H. pylori selective supplement (Dent) and nutritional supplement (Vitox)

To a bottle of Dent, 2 ml of sterile distilled water was added and mixed gently. The solution was used the same day. Vitox was prepared by mixing the provided solvent and the Vitox powder. All were prepared according to the manufacturers' instructions.

Transport medium (5% BHIA + 5% FBS)

The above is the semi-solid agar for transportation of gastric biopsies. Five grams of BHIA was weighed, suspended in 95 ml of sterile distilled water, dissolved by boiling, and subsequently autoclaved at 121°C for 15 minutes. After autoclaving, the medium was cooled to 45°C in a water-bath and gently mixed with a 5 ml aliquot of the inactivated FBS. Aliquots of 500 µl were dispensed into 1.5 ml tubes and stored at 4°C in a refrigerator until use. The transport medium was used within 1 week from the preparation date. A sterility control was done on one tube from the preparation under aerobic and micro-aerophilic conditions, at 37°C for 24 hours.

Non-selective culture media: Colombia blood agar (CBA) + 7% FBS and BHIA + 7% FBS plates (CBA + 7% FBS and BHIA + 7% FBS)

CBA (19.5±0.1 g) and BHIA (23.5±0.1 g) (Oxoid, UK) were each weighed and put in separate 500 ml bottles. Both media were suspended in 500±0.5 ml sterile distilled water and fully dissolved by boiling. These bottles were labeled 'CBA + 7% FBS' and 'BHIA + 7% FBS' respectively and autoclaved at 121°C for 15 minutes. After autoclaving, the media were cooled to 45°C in a water-bath and gently mixed with 35 ml aliquots of the inactivated FBS and the Vitox supplement. The media were poured (approximately 25 ml per plate), left to solidify, and dried at room temperature for 2 hours before being stored at 8°C in a refrigerator until use. The plates were used within 1 week from the preparation date. Sterility control on 2 plates from each pack of 20 plates was done at 37°C for 24 hours, 1 under aerobic and 1 under micro-aerophilic conditions.

Selective culture media: CBA + 7% FBS + Dent, and BHI agar + 7% FBS + Dent plates (CBAD + 7% FBS and BHIAD + 7% FBS)

(CBAD = Colombia blood agar with Dent selective supplement containing vancomycin, trimethoprim, cefsulodin and amphotericin B; BHIAD = brain-heart-infusion agar with Dent selective supplement containing vancomycin, trimethoprim, cefsulodin and amphotericin B.)

CBA (19.5±0.1 g) and BHIA (23.5±0.1 g) (Oxoid, UK) were each weighed and put in separate 500 ml bottles. Both media were suspended in 500±0.5 ml of sterile distilled water and were fully dissolved by boiling. These bottles were labelled 'CBAD + 7% FBS' and 'BHIAD + 7% FBS' respectively, autoclaved at 121°C for 15 minutes, cooled down to 45°C in a water-bath, and gently mixed with the inactivated FBS; thereafter, Dent and Vitox were added and mixed by gently rolling the bottle. The media were poured (approximately 25 ml per plate), left to solidify, and dried at room temperature for 2 hours before being stored at 8°C in a refrigerator until use. The plates were used within 1 week from the preparation day. Sterility control at 37°C for 24 hours on two plates from each pack of 20 plates was done; 1 under aerobic and 1 under micro-aerophilic conditions.

H. pylori growth

The biopsies were transferred by sterile 1 000 µl pipette to clean sterile tubes, where they were completely crushed using the pipette tip; 200 µl of BHI broth, enriched with 5% FBS, was added and uniformly mixed. Next, 100 µl of the ruptured and homogenised material was aseptically transferred to each of the appropriately labelled plates, one selective and the other non-selective, using a disposable 10 µl sterile inoculating loop. The inoculum was aseptically spread on the surface of the plates. Inverted, the plates were placed in a 2.5 l jar. A Campy Micro-aerophilic kit was added, and the jar immediately closed and incubated undisturbed at 37°C for 3 days. After 3 days, the plates were removed and visually inspected. When the colonies appeared too small, a new Campy Micro-aerophilic kit was placed in the 2.5 l jar and the plates re-incubated immediately for a further 2 days. If growth was not satisfactory after 5 days, further incubation was done up to 7 days with a new Campy Micro-aerophilic kit until the colonies were well visible. *H. pylori* identification was confirmed by colony morphology, Gram-stain, and oxidase, urease and catalase positivity. Old cultures were a mixture of short rods and coccoids, while young cultures were curved rods. Subcultures were done on antibiotic-free media. Pure culture was harvested by transferring 200 µl phosphate-buffered saline (PBS) onto the plate. The pure bacteria growth on the plate was emulsified in the PBS using a sterile inoculating loop. The suspension was then transferred to cryotubes containing 500 µl BHI broth enriched with 20% glycerol and 5% FBS, and stored at -80ºC.

54

H. pylori sensitivity testing

A frozen vial of the correctly identified *H. pylori* was thawed at room temperature and mixed by pippeting up and down with a micropipette. This sample was used for subculture. Young, pure cultures were harvested by transferring 200 µl PBS onto the culture plate and the bacteria emulsified into a suspension using a sterile inoculating loop. The suspension was collected into a sterile tube and a McFarland's turbidity standard no. 3 made for use in susceptibility testing. The inoculum was aseptically spread over the entire portion of the surface of the sensitivity test plate, and allowed to stand at room temperature for 10 minutes before the antibiotic E-test strips for clarithromycin (0.016-256); metronidazole (0.002-32) and amoxicillin (0.016-256) were applied. Inverted inoculated plates were then placed into the jar. One envelope of Campy Micro-aerophilic gas kit was put into the 2.5 l jar following the manufacturer's instructions. The jar was incubated at 37°C for 24 hours, and then removed and inspected for satisfactory *H. pylori* growth. If the growth were insufficient, the plates were immediately put back into the jar with a new CampyGen Micro-aerophilic gas kit, and incubation continued at 37°C for a further 12 hours. When growth was satisfactory, the minimum inhibiting concentration (MIC) for each antibiotic was read as recommended by the National Committee on Clinical Laboratory Standards/Clinical and Laboratory Standards Institute (NCCLS/CLSI) standards.

Results

Of the 696 patients with dyspepsia who were screened, 66.1% were adults and 33.9% were children. The prevalence of *H. pylori* infection in the adult segment was 54.8%; and 73% in children (Fig. 1). Culture was attempted on 70 biopsies; of these, 65 (92.8%) were successful.

Prevalence of *H. pylori* infection was 65% in African immigrants, 25% in Asian immigrants, 56% in rural Kenyan Africans, 62% in urban Kenyan Africans, and 58% among urban Kenyan Asians. Gastro-esophageal reflux disease (GERD) accounted for 201 (28.9%) of the cases, gastric polyps 3 (0.4%), atrophic mucosa 4 (0.6%), gastric cancer 6 (0.9%), gastritis 452 (64.9%), duodenal ulcer 12 (1.7%), and gastric ulcer 18 (2.6%) (Fig. 2). GERD was more common in Kenyan female patients (56 - 65.6%) than males.

Gastric and duodenal ulcers occur with equal frequency in all racial groups. Despite a high prevalence (67.5%) of *H. pylori* in Kenya, the complicated disease outcomes (such as gastric cancer) are relatively low in this series (Table I).

Results of susceptibility testing for metronidazole (MZ), amoxicillin (AX) and clarithromycin (CL) and the total number of *H. pylori* bacilli susceptible at different concentration of these drugs are summarised in Fig. 3. MIC levels of <8 µg/ml for MZ and AX, and <2 µg/ml for CL, were interpreted as sensitive.
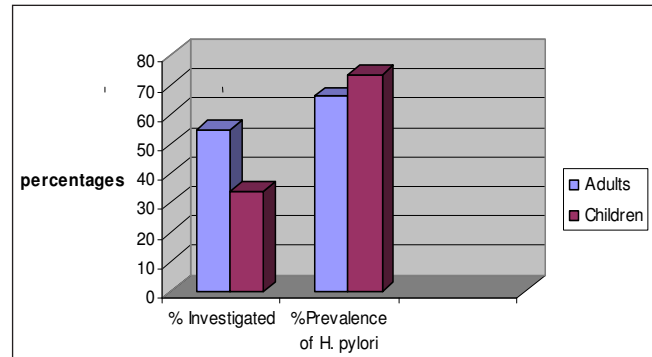

Fig. 1. Percentage of cases investigated and corresponding H. pylori *prevalence.*

GERD = gastro-oesophageal reflux disease; POLY = gastric polyp; AM = atrophic mucosa; CA = gastric cancer; DU = duodenal ulcer; GU = gastric ulcer.
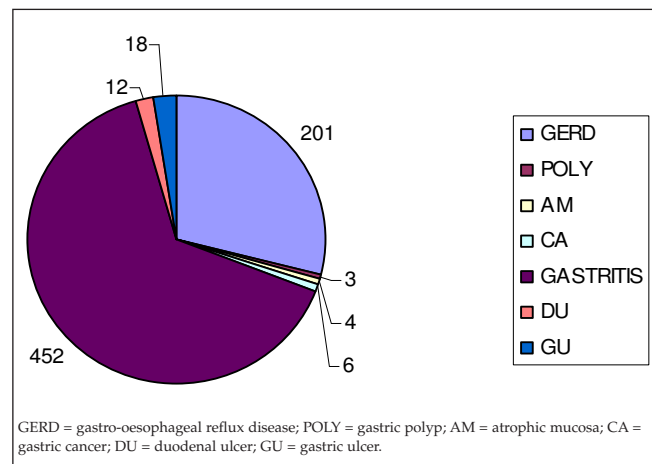
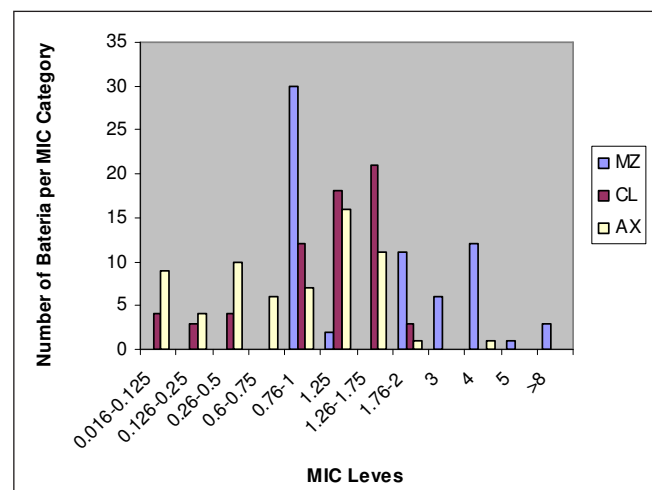*Fig. 2. Major endoscopic findings.*

Fig. 3. Antibiotic sensitivity profiles.

All the *H. pylori* that we investigated were largely sensitive to clarithromycin (100%), amoxicillin (100%) and metronidazole (95.4%). However, there was occasional resistance to metronidazole (4.6%). The 3 antibiotics showed no significant difference in activity (mean±SD: MZ 5±8.6, CL 5±7.3, AX 5±5.3, $p=1$). Both CBA and BHIA effectively supported *H.*

Table I. Racial/regional groupings and disease outcome								
Race/regional background	*N* (%)	GERD	POLY	AM	CA	Gastritis	DU	GU
African immigrant	49 (7)	12 (24.5)	0 (0)	0 (0)	1 (2)	34 (69.4)	1 (2)	1 (2)
Asian immigrant	14 (2)	7 (50)	0 (0)	0 (0)	0 (0)	6 (42.9)	0 (0)	1 (7)
Kenyan-African rural	186 (26.7)	63 (33.9)	1 (0.5)	4 (2.2)	2 (1.1)	106 (57)	4 (2.2)	6 (3.2)
Kenyan-African urban	319 (45.8)	69 (21.6)	0 (0)	0 (0)	3 (0.9)	237 (74.3)	4 (1.3)	6 (1.9)
Kenyan-Asian urban	69 (9.9)	25 (36.2)	2 (2.9)	0 (0)	0 (0)	37 (53.6)	2 (2.9)	3 (4.3)
Others	59 (8.5)	25 (42.4)	0 (0)	0 (0)	0 (0)	32 (54.2)	1 (1.7)	1 (1.7)
Total	696	201 (28.4)	3 (0.4)	4 (0.6)	6 (0.9)	452 (64.9)	12 (1.7)	18 (2.6)

GERD = gastro-oesophageal reflux disease; POLY = gastric polyp; AM = atrophic mucosa; CA = gastric cancer; DU = duodenal ulcer; GU = gastric ulcer.

pylori growth achieved in an average of 36 hours for primary isolations and 24 hours for subcultures (data not shown).

Discussion

Prevalence of *H. pylori* was found to be 67.5% in all age groups (73.3% in children and 54.8% in adults), similar to the findings of Shmuely *et al*,[1] who documented 60 - 73% in all age groups in dyspeptic patients. However, this finding differs with studies in other African countries which found *H. pylori* prevalence levels of >90%.[9-13] Gastritis accounted for the largest proportion (64.9%) of the *H. pylori*-associated pathologies, as found elsewhere in Africa.[9] It has been noted that, despite the prevalence of a virulent strain and high prevalence of *H. pylori*, the associated pathology (duodenal ulcer, gastric ulcer and gastric cancer) is variable and often low-incidence, and its distribution in sub-Saharan Africa does not parallel *H. pylori* prevalence in the population.[14,15] This phenomenon was strongly supported by the findings of our study, where atrophic mucosa represented 0.6%, gastric cancer 0.9%, duodenal ulcer 1.7%, and gastric ulcer 2.6% of all observed pathologies, despite the high prevalence (67.5%) of *H. pylori* infection, which implies that, in this decade, the old *status quo* still stands.

We found no difference in the distribution of *H. pylori* pathologies among the different ethnic inhabitants of Kenya. In 2001, Segal *et al*.[14] reported that, despite the presence of virulent strains in Africa, associated pathologies are not in tandem. He suggested that host immune response may be a factor in protecting against development of gastric cancer. Our findings seem to suggest otherwise. If host immune response were a factor, we should see a varied distribution of pathologies in our study population of different ethnic groups. Therefore, we suggest that the development of significant clinical pathology in *H. pylori* infection depends on a combination of complex factors, possibly host genetics, environment, pathogens and age of the host.

All the *H. pylori* investigated in this study were largely sensitive to clarithromycin (100%), amoxicillin (100%) and metronidazole (95.4%). There was, however, occasional resistance to metronidazole (4.6%). The 3 antibiotics showed

no significant difference in activity (mean±SD: MZ 5±8.6, CL 5±7.3, AX 5±5.3, *p*=1). Susceptibility rates in Cameroon were found to be 55.3% for clarithromycin, 14.4% for amoxicillin and 6.8% for metronidazole.[13] The documented resistance of amoxicillin in Africa signifies the emergence of *H. pylori* strains resistant to this key component in *H. pylori* treatment protocols. Up to the year 2000, amoxicillin-resistant *H. pylori* strains had rarely been detected, with a total of only 14 reported.[16] Significantly, complete loss of the resistant phenotype was observed after these strains were stored at -80°C, owing to the process of freezing and thawing. Only one amoxicillin-resistant *H. pylori* strain had been isolated, in the Netherlands, in which amoxicillin resistance remained stable after repeated cycles of freezing and culture.[17] The MIC for this strain was 8 μg/ml, which is relatively low. A survey is therefore necessary to confirm the presence of amoxicillin-resistant *H. pylori* strains in Africa. Amoxicillin-resistant *H. pylori* strains in circulation are a threat to public health, as this resistance trait may be transferable to amoxicillin-susceptible strains,[17] presumably owing to DNA exchange by transformation or a conjugation-like mechanism.[18] Our study, however, shows a high susceptibility to amoxicillin among *H. pylori* strains.

The media we described support *H. pylori* growth in an average of 36 hours for primary isolations and 24 hours for subcultures, with an isolation rate of 92.8% (65/70) whereas an isolation rate of 47% (31/66) in Kenya was achieved in growth using Modified Thayer-Martin agar after 4 days of incubation.[4] The medium used in this study is therefore considered superior and can be used for enhanced cultivation of *H. pylori* and susceptibility testing.

Lastly, the great diversity of ethnic and racial groups in Kenya, and the high prevalence of *H. pylori* but the low prevalence of gastric carcinoma, call for closer examination of *H. pylori* in different regions and ethnic groups within Kenya as well as within surrounding regions. This investigation can provide new data regarding both the diversity of *H. pylori* as well as the co-migration of *H. pylori* and its human hosts.

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