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GASTRIC FLORA CULTURED ON STANDARD MEDIA FOR *HELICOBACTER PYLORI*

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Abstract: The aim of the study was to assess microorganisms growing on standard media for *Helicobacter pylori* culture. In the majority of the population studied, apart from *H. pylori* there were extra 1-3 colonies of 29 bacteria and 2 yeasts; the most common microorganisms cultured besides *H. pylori* were *Streptococcus salivarius*, *Staphylococcus epidermidis*, and *Corynebacterium spp*. The low percentage of failed *H. pylori* culture and the simultaneous success of culture for other microorganisms suggests that other microorganisms occurring in the stomach only slightly inhibit *H. pylori* growth under laboratory conditions.

Key words: bacterial culture, gastric flora, gastric mucosa specimens, *Helicobacter pylori*.

Introduction

There are 300-500 various bacteria in the gastrointestinal lumen [1]. The majority of them are well-adjusted to the ecosystem and do not cause any harm to the body. The stomach is the part of the gastrointestinal tract where the population of bacteria is not too large. This is related to the low pH inside; most bacteria need a pH close to neutral. However, some of them, which exhibit urease activity, are capable of surviving in the acidic environment for up to several hours. These bacteria break down urea to ammonia and create an area of increased pH; *Helicobacter pylori* (*H. pylori*) is one of them [2]. When it is present in the stomach, it causes inflammation of the mucosa, and it promotes the formation of stomach and duodenum ulcers and also gastric cancer [3]. The urease activity has been used in diagnostics of *H. pylori* stomach infection; urease activity is the basis for two tests which still play the most important role in clinical practice: *Campylobacter*-like organisms (CLO) test and urea breath test. The sensitivity and specificity of both tests are at the level of 80-95% [4,5]. However, a certain number of false positive and false negative results must be taken into account in the assessment of stomach infection with *H. pylori*. False positive results are related to the presence of urease-positive bacteria other than *H. pylori* in the stomach [2], while false negative results to a low number of *H. pylori* bacteria or their low urease activity, e.g. due to the treatment with drugs inhibiting the gastric secretion of hydrogen ions [6].

The aim of the study was to assess the occurrence of gastric flora other than *H. pylori* in endoscopic specimens taken from patients with a positive result of urease test (CLO test).

Materials and methods

158 patients with a positive result of a CLO test participated in the study (Table 1). Some of them took proton pump inhibitors (PPIs) (10 mg omeprazole, 20 mg pantoprazole and 15 mg lansoprazole or more daily for at least

3 days before the inclusion), some were smokers (smoking at least 5 cigarettes daily during the month preceding the inclusion) and alcohol consumers (drinking at least 25 g of pure alcohol weekly during the month preceding the inclusion).

Table 1: Patient Data.

age (median, range)	53 (19-79)
gender (M/F)	68/90
smokers	43(27.2%)
alcohol users	43(27.2%)
PPI users	46(29.1%)
Diagnosis:	
dyspepsia	114(72.1%)
duodenal ulcer disease	42(26.6%)
gastric ulcer disease	2(1.3%)

The specimens were collected from the prepyloric and the gastric body regions during diagnostic gastroscopy of patients from the Internal Medicine and Gastroenterology Department District Hospital of Białystok; one for the CLO test, one for culture and two for the microscopic examination. For inclusion in the study patients needed to be in good health, have a normal range of laboratory tests and not have used antibiotics for at least one month.

The CLO test was prepared at the Department of Physiology Medical University of Białystok in accordance with the method of Marshall et al. [7]; the test sensitivity and specificity as compared to the histological examination, culture, and stool test were 84.3% and 88.4%, 87.5% and 83.5%, and 75.4% and 87.5%, respectively [8]. The CLO test was evaluated 2 hours after the collection of specimens. The endoscopic specimens of the mucosa taken for the microscopic assessment were collected into buffered formalin and subjected to routine processing. Microscopic evaluation was performed by two experienced pathologists.

Endoscopic specimens of the gastric mucosa taken for culture were collected to a transport medium (Portagerm pylori, bioMerieux) and were delivered to a microbiology laboratory within 3 hours. The bacteria were cultured using the Columbia Agar medium with a 5-7% addition of sheep blood and the Agar Pylori selective medium (bioMerieux) for 7-14 days at a temperature of 37°C under microaerophilic conditions. Morphological features of colonies, the image of the bacteria in the microscopic examination (Gram staining) and the ability of the bacteria to produce urease, oxidase and catalase were taken into account in the identification of the *H. pylori* bacteria. The identification of other cultured microorganisms was conducted on the basis of the criteria used for the identification of the *H. pylori* bacteria, as well as using the ID 32 E, ID 32 GN, rapid ID 32 Strep, ID 32 Staph, Api NH, Api Candida kits (bioMerieux).

The results were evaluated statistically using χ^2 test (Statistica 2008). The differences were considered to be statistically significant at $p<0.05$.

Results

Out of 158 patients with a positive result of the CLO test, in 13/158 (8.2%) no *H. pylori* bacteria were cultured, while in 8/158 (5.1%) only *H. pylori* bacteria were cultured but no other microorganisms (Table 2). In total, 31 microorganisms, 29 bacteria and 2 yeasts were cultured, apart from *H. pylori* (Table 3). The group of 150 patients in whom microorganisms other than *H. pylori* were identified, 97/150 (64.7%) had one additional microorganism, 47/150 (31.3%) two microorganisms and 6/150 (4.0%) three microorganisms. Forty out of 150 (26.7%) patients in whom microorganisms other than *H. pylori* were cultured took PPIs. Both in subjects taking and not taking PPIs, the percentage of those with two or three microorganisms cultured apart from *H. pylori* was 40.0% and 5.0% vs. 28.2% and 3.6%, respectively. Similar values were obtained for smokers and non-smokers (36.6% and 2.4% vs. 29.4% and 4.6%, respectively) and alcohol users and alcohol non-users (31.7% and 0% vs 31.2% and 5.5%, respectively). The microorganisms most frequently cultured from endoscopic specimens from the gastric mucosa, apart from *H. pylori* were *Streptococcus salivarius*, *Staphylococcus epidermidis* and *Corynebacterium spp.* (Table 3). The use of PPIs, smoking or drinking alcohol changed the frequency of occurrence of some microorganisms in the gastric mucosa, but not from the most frequently occurring group of microorganisms (Table 4-6). *Streptococcus oralis, Enterococcus faecalis, Streptococcus gr. viridans* and *Moraxella spp.* occurred a few times more frequently in patients taking PPIs, while *Rothia mucilagenosa, Escherichia coli* and *Staphylococcus warnerii* occurred a few times less frequently (a significant difference was found only in the case of *Enterococcus faecalis*, $p=0.024$)(Table 4). *Corynebacterium spp., Actinobacillus spp., Kingella spp.* and *Staphylococcus aureus* were found a few times more frequently in smokers, while *Gemella morbillorum, Eikenella corrodens, Escherichia coli, Streptococcus mitis, Staphylococcus wernerii, Streptococcus gr. viridans* were found a few times less frequently (Table 5). *Corynebacterium spp., Micrococcus luteus, Escherichia coli, Staphylococcus warnerii* and *Streptococcus sanguis* were found a few times more frequently in alcohol users, while *Gemella morbillorum, Neisseria spp., Streptococcus oralis, Micrococcus spp.* and *Candida albicans* were found a few times less frequently (a significant difference was found only in the case of *Staphylococcus warnerii*, $p=0.038$) (Table 6). Out of 5 patients with a positive result of the CLO test and a negative result from both the

Table 2: Characteristics of Subjects with Positive CLO Test.

Histology	***H. pylori* Culture**	**Culture of other microorganisms**	**n = 158(100%)**
+	+	+	128(81.0%)
+	+	-	7(4.4%)
-	+	+	9(5.7%)
-	+	-	1(0.6%)
-	-	+	5(3.2%)
+	-	+	8(5.1%)

Table 3: The Occurrence of Microorganisms other than *H. pylori* in the Gastric Mucosa of Subjects with Positive CLO Test.

	(n = 150)
Streptococcus salivarius	33(22.0%)
Staphylococcus epidermidis	20(13.3%)
Corynebacterium spp.	14(9.3%)
Neisseria spp.	13(8.7%)
Gemella morbillorum	12(8.0%)
Actinobacillus spp.	10(6.7%)
Rothia mucilaginosa	9(6.0%)
Lactobacillus spp.	9(6.0%)
Eikenella corrodens	9(6.0%)
Micrococcus luteus	9(6.0%)
Kingella spp.	8(5.3%)
Streptococcus oralis	7(4.7%)
Streptococcus mitis	6(4.0%)
Enterococcus faecalis	6(4.0%)
Streptococcus gr. viridans	6(4,0%)
Escherichia coli	5(3.3%)
Staphylococcus xylosus	5(3.3%)
Staphylococcus warnerii	4(2.7%)
Staphylococcus aureus	4(2.7%)
Streptococcus sanguis	3(2.0%)
Moraxella spp.	3(2.0%)
Micrococcus spp.	3(2.0%)
Candida albicans	3(2.0%)
Streptococcus mutans	2(1.3%)
Candida spp.	1(0.7%)
Leukonostoc spp.	1(0.7%)
Rothia dentocariosa	1(0.7%)
Pseudomonas aeruginosa	1(0.7%)
Streptococcus equinus	1(0.7%)
Streptococcus parasanguinis	1(0.7%)
Neisseria sicca	1(0.7%)

Table 4: The Occurrence of Microorganisms other than *H. pylori* in the Gastric Mucosa of CLO Test Positive Subjects Taking and Non-taking PPIs.

	PPI users (n =40)	**PPI non-users (n =110)**
Streptococcus salivarius	10(25.0%)	23(20.9%)
Staphylococcus epidermidis	6(15.0%)	14(12.7%)
Corynebacterium spp.	3(7.5%)	11(10.0%)
Neisseria spp.	3(7.5%)	10(9.1%)
Gemella morbillorum	3(7.5%)	9(8.2%)
Actinobacillus spp.	2(5.0%)	8(7.3%)
Rothia mucilaginosa	0	9(8.2%)
Lactobacillus spp.	2(5.0%)	7(6.4%)
Eikenella corrodens	3(7.5%)	6(5.5%)
Micrococcus luteus	3(7.5%)	6(5.5%)
Kingella spp.	3(7.5%)	5(4.5%)
Streptococcus oralis	4(10.0%)	3(2.7%)
Streptococcus mitis	2(5.0%)	4(3.6%)
Enterococcus faecalis	4(10.0%)	2(1.8%) *
Streptococcus gr. viridans	3(7.5%)	3(2.7%)
Escherichia coli	0	5(4.5%)
Staphylococcus xylosus	1(2.5%)	4(3.6%)
Staphylococcus warnerii	0	4(3.6%)
Staphylococcus aureus	0	3(2.7%)
Streptococcus sanguis	0	3(2.7%)
Moraxella spp.	2(5.0%)	1(0.9%)
Micrococcus spp.	0	3(2.7%)
Candida albicans	0	3(2.7%)
Streptococcus mutans	1(2.5%)	2(1.8%)
Candida spp.	1(2.5%)	1(0.9%)
Leukonostoc spp.	0	1(0.9%)
Rothia dentocariosa	1(2.5%)	1(0.9%)
Pseudomonas aeruginosa	0	1(0.9%)
Streptococcus equinus	0	1(0.9%)
Streptococcus parasanguinis	0	1(0.9%)
Neisseria sicca	0	1(0.9%)

* p=0.024

culture and the histology, *Neisseria spp.* were cultured in 3 patients, while *Staphylococcus epidermidis* and *Candida albicans* in the two other patients, respectively.

Discussion

The human stomach, due to the low pH inside is regarded as an unfavourable environment for the growth of microorganisms. Only some bacteria which cross the gastric mucous covering the epithelium can live there causing

Table 5: The Occurrence of Microorganisms other than *H. pylori* in the Gastric Mucosa of CLO Test Positive Smokers and Non-smokers.

	smokers (n=41)	**non-smokers (n=109)**
Streptococcus salivarius	7(17.1%)	26(23.9%)
Staphylococcus epidermidis	4(9.6%)	15(13.8%)
Corynebacterium spp.	7(17.1%)	6(5.5%)
Neisseria spp.	4(9.6%)	6(5.5%)
Gemella morbillorum	2(4.9%)	10(9.2%)
Actinobacillus spp.	5(12.2%)	5(4.6%)
Rothia mucilaginosa	3(7.3%)	6(5.5%)
Lactobacillus spp.	2(4.9%)	7(6.4%)
Eikenella corrodens	1(2.4%)	8(7.3%)
Micrococcus luteus	4(9.6%)	5(4.6%)
Kingella spp.	5(12.2%)	3(2.8%)
Streptococcus oralis	2(4.9%)	5(4.6%)
Streptococcus mitis	1(2.4%)	6(5.5%)
Enterococcus faecalis	2(4.9%)	4(3.7%)
Streptococcus gr. viridans	1(2.4%)	5(4.6%)
Escherichia coli	1(2.4%)	5(4.6%)
Staphylococcus xylosus	2(4.9%)	3(2.8%)
Staphylococcus warnerii	0	5(4.6%)
Staphylococcus aureus	3(7.3%)	1(0.9%)
Streptococcus sanguis	0	3(2.8%)
Moraxella spp.	1(2.4%)	2(1.8%)
Micrococcus spp.	1(2.4%)	2(1.8%)
Candida albicans	1(2.4%)	1(0.9%)
Streptococcus mutans	0	2(1.8%)
Candida spp.	2(4.9%)	1(0.9%)
Leukonostoc spp.	0	2(1.8%)
Rothia dentocariosa	1(2.4%)	0
Pseudomonas aeruginosa	1(2.4%)	0
Streptococcus equinus	0	1(0.9%)
Streptococcus parasanguinis	1(2.4%)	0
Neisseria sicca	1(2.4%)	0

Table 6: The Occurrence of Microorganisms other than *H. pylori* in the Gastric Mucosa of CLO Test Positive Alcohol Users and Non-users.

	alcohol users (n=43)	**alcohol non-users (n=107)**
Streptococcus salivarius	7(16.3%)	26(24.3%)
Staphylococcus epidermidis	7(16.3%)	13(12.1%)
Corynebacterium spp.	6(13.9%)	8(7.5%)
Neisseria spp.	1(2.3%)	12(11.2%)
Gemella morbillorum	1(2.3%)	11(10.3%)
Actinobacillus spp.	3(7.0%)	7(6.5%)
Rothia mucilaginosa	2(4.6%)	7(6.5%)
Lactobacillus spp.	2(4.6%)	7(6.5%)
Eikenella corrodens	2(4.6%)	7(6.5%)
Micrococcus luteus	4(9.3%)	5(4.7%)
Kingella spp.	2(4.6%)	6(5.6%)
Streptococcus oralis	1(2.3%)	6(5.6%)
Streptococcus mitis	2(4.6%)	4(3.7%)
Enterococcus faecalis	1(2.3%)	5(4.7%)
Streptococcus gr. viridans	1(2.3%)	4(3.7%)
Escherichia coli	3(7.0%)	2(1.9%)
Staphylococcus xylosus	1(2.3%)	4(3.7%)
Staphylococcus warnerii	3(7.0%)	1(0.9%)*
Staphylococcus aureus	1(2.3%)	2(1.9%)
Streptococcus sanguis	2(4.6%)	1(0.9%)
Moraxella spp.	1(2.3%)	2(1.9%)
Micrococcus spp.	0	3(2.8%)
Candida albicans	3(2.8%)	3(2.8%)
Streptococcus mutans	1(2.3%)	1(0.9%)
Candida spp.	1(0.9%)	1(0.9%)
Leukonostoc spp.	1(2.3%)	0
Rothia dentocariosa	1(0.9%)	1(0.9%)
Pseudomonas aeruginosa	1(0.9%)	1(0.9%)
Streptococcus equinus	1(0.9%)	1(0.9%)
Streptococcus parasanguinis	1(2.3%)	0
Neisseria sicca	1(0.9%)	1(0.9%)

* p=0.038

the mucosal inflammation; *H. pylori* is an example [9]. Although a considerable number of microorganisms found in endoscopic specimens of the gastric mucosa can live in an oxygen-poor atmosphere, the aerobic bacteria can be encountered there, too (*Micrococcus*). As the results of the present study show, other microorganisms than *H. pylori* may occur in the stomach, and their source is usually the oral cavity [10–12]. However, the identified bacteria did not reflect the actual image of the whole gastric mucosal flora, since the culture was conducted under microaerophilic conditions favourable for the development of microorganisms tolerating oxygen-poor atmosphere.

Various microorganisms can grow in the media used for culturing *H. pylori* (this concerns mainly Columbia Agar with the addition of 5-7% sheep blood) [13]. By qualifying for the study only patients with a positive result of the urease test (CLO test), patients with relatively small population of *H. pylori* bacteria in the stomach or the low urease activity were eliminated from the study on a preliminary basis. On the other hand, the use of PPIs, which was not an exclusion criterion, could lead to an increase in the mucosa of several other microorganisms, other than *H. pylori*. A small percent of negative *H. pylori* culture in patients with a positive result of the CLO test does not have to unambiguously prove the inhibition of *H. pylori* growth by other bacteria occurring in the stomach. In half of the cases mentioned above, a positive result of the CLO test was not confirmed by histological examination either, which implies the presence in the mucosa some microorganisms other than *H. pylori* having urease activity. Among these bacteria identified in the endoscopic specimens characterized by such activity were *Corynebacterium, Staphylococcus, Streptococcus, Micrococcus,* and *Enterococcus*. Although the urease activity of these bacteria is lower than *H. pylori*, it cannot be ruled out that it could affect the result of the CLO test [14, 15].

Stomach infection with *H. pylori* does not have an influence on the other bacteria occurring in the stomach [13]. *H. pylori* is the predominant microorganism found in the stomach, while *Streptococcus mitis* is ranked second [13]. In our study, *Streptococcus salivarius* was the second most frequent bacterium after *H. pylori*; *Streptococcus mitis* occurred less frequently. Unfortunately, due to the culturing in oxygen-poor atmosphere, our study eliminated some microorganisms normally occurring in the stomach. Moreover, it should be taken into account that a considerable number of bacteria occurring in the stomach cannot be cultured at all [13].

PPIs (omeprazole, pantoprazole, lansoprazole) are the basic treatment for conditions connected with the secretion of gastric hydrogen ions. This medication not only increases the pH level inside the stomach creating favourable conditions for the development of some bacteria [16], but it also exhibits antibacterial activity in relation to the number of microorganisms [15,17–19]; it inhibits the urease activity of *H. pylori* and does not affect the urease activity of other urease-positive bacteria [6,20,21]. Microorganisms which we cultured from endoscopic specimens under microaerophilic conditions did not exhibit significant qualitative differences between the group of patients who received and did not receive PPIs; such differences were rather quantitative in nature. The lack of qualitative differences could have been influenced by the duration of the use of this medication and their doses. Differences with a similar character to those found in PPIs users were found in smokers and alcohol users. This may mean that the population of gastric microorganisms is not only influenced by medication inhibiting the secretion of gastric hydrogen ions but also in some extend by other factors.

The elimination of *H. pylori* bacteria from the stomach reduces the inflammation of the mucosa but it does not eliminate it completely [22]. After the eradication of *H. pylori*, the stomach is not deprived of bacteria, bacteria other than *H. pylori* may, together with other factors such as smoking, contribute to the persistence of inflammation, although the intensity of inflammation is considerably reduced in such cases [22].

Conclusions

By culturing *H. pylori* from endoscopic specimens of gastric mucosa one may expect some difficulties resulting from the presence of other microorganisms tolerating or preferring microaerophilic conditions for their growth, i.e. microorganisms which grow relatively well on standard media for *H. pylori*. However, the low percentage of subjects with negative culture of *H. pylori* in the case of confirmation of its presence in the stomach by other methods implies that other gastric flora only slightly inhibit its growth in culture under microaerophilic conditions.

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