Pol. J. Appl. Sci., **2017**, *3*, 16-19

THE USE OF IMMUNOLOGICAL METHOD FOR IDENTIFICATION OF *HELICOBACTER PYLORI* IN CULTURE

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Abstract: The aim of the study was to establish whether the immune method detecting *Helicobacter pylori* antigens can be used for the identification of *H. pylori* from a culture. Bacteria were cultured from endoscopic specimens of gastric mucosa from 378 patients. A positive result of *H. pylori* culture was obtained in 166 patients (43.9%), while the presence of *H. pylori* antigens was obtained in 164 (98.8%). The classical method for *H. pylori* identification from a culture is characterized by high accuracy. Therefore, a positive result of the test for the presence of *H. pylori* antigens from the culture could only be additional evidence confirming the culture results in selected cases.

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

Introduction

There are a number of methods for detecting stomach infection with *H. pylori* [1–6]. One of them is the culture of bacteria from endoscopic specimens. Since the culture is expensive and labour-consuming, it is rarely used in diagnostics, at least in Poland. In accordance with the recommendation of the European and Polish *Helicobacter pylori* Study Groups, bacterial culture combined with an assessment of drug sensitivity should be performed when two successive attempts of *H. pylori* eradication therapy have ended in failure [1, 2].

Each bacterial colony grown in a Petri dish usually has a very characteristic appearance. Regardless of this a model for its further identification is defined. For *H. pylori* a direct Gram stained preparation is used, and its urease, catalase and oxidase activity are assessed [5]. Sometimes the colony may have an atypical appearance, but the microscopic preparation and tests evaluating the urease, catalase and oxidase activity exhibit positive results. Therefore, it is proposed that another technique, molecular biology, should be used in the identification of *H. pylori* bacteria from a culture, instead of the classical identification tests [5].

The aim of the study was to establish whether the immune method which has historically been used for detecting *H. pylori* antigens in faeces, dental plaque, saliva, and gastric mucosa [7–14] could be used for identification of *H. pylori* from a culture.

Materials and methods

Three hundred and seventy eight patients participated in the study (Table 1). The specimens one for *Campylobacter*-like organism (CLO) test, one for culture and two for microscopic examination were collected from the prepyloric and the gastric body regions during diagnostic gastroscopy of patients of the Internal Medicine and Gastroenterology Department District Hospital in Bialystok. The inclusion criteria involved patient's good health, the normal range of basic laboratory tests, and no treatment with antibiotics for at least one month before recruitment.

The urease test (CLO test) was prepared at the Department of Physiology, Medical University of Białystok in accordance with the Marshall et al. [15] method; the test sensitivity and specificity were 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 [12].

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Table 1: Patients' characteristics.

Age (median, range)	56 (18-81)
Gender (M/F)	167 / 211
Smokers	93 (24.6%)
Alcohol users	101(26.7%)
Diagnosis: **Dyspepsia** **Peptic ulcer disease**	 287 (75.9%) 91 (24.1%)

The endoscopic specimens of the mucosa for the microscopic assessment were collected into buffered formalin and subjected to routine processing. Microscopic preparations were assessed by two experienced pathologists.

The endoscopic specimens of the gastric mucosa taken for culture were collected in a transport container (Portagerm pylori, bioMerieux) and after preliminary processing were inoculated into the Columbia Agar medium with the addition of 5-7% of sheep blood and the Agar Pylori selective medium (bioMerieux). The culture was kept at a temperature of 37°C under microaerophilic conditions for 5-14 days. The following elements were taken into account in the identification of the *H. pylori* bacteria from the culture: the morphological features of colonies, the image of bacteria in microscopic examination (Gram staining) and the ability of the bacteria to produce urease, oxidase and catalase. A stain test (Becton Dickinson and Company, USA) was used for the assessment of bacterial oxidase; if oxidase is present, it changes the colour to dark purple in a few seconds. The test for the presence of catalase involved adding bacteria obtained from the culture to a drop of 3% H_2O_2; gas bubbles released in a few seconds confirmed a positive result. For the analysis of urease activity the same test was used as for the assessment of endoscopic specimens of gastric mucosa. The identification of other cultured bacteria was based on the ID 32E, ID 32GN, rapid ID 32Strep, ID 32Staph and API NH kits (bioMerieux).

Bacteria which met the identification criteria for *H. pylori* (n=166) as well as other bacteria cultured from endoscopic specimens of gastric mucosa which did not meet the identification criteria for *H. pylori* were analysed for the presence of *H. pylori* antigens (Table 2, 3). Bacteria from reference strain cultures were also tested for the presence of *H. pylori* antigens (Table 4).

A test (IDEIA HpStAR; OXOID) for the assessment of the presence of *H. pylori* antigens in faeces was used to determine *H. pylori* antigens from the culture. For this purpose, a suspension of bacteria in saline was prepared with an inoculum of 10^8 cfu/ml [16]. Next, a solvent (100 μl) included in the kit by the manufacturer was added to 100 μl of the deposit remained after centrifuging this suspension (at 5000 rpm for 5 minutes). Further stages of the test were performed in accordance with the manufacturer's instruction. During the first stage, the prepared supernatant was transferred to a microtitration plate in which monoclonal anti-*H. pylori* antibodies had been placed in special microwells prepared by the manufacturer. Additionally, monoc-

Table 2: The tests results for *H. pylori*; A – culture negative (n=212), B – culture positive (n=166).

A

CLO-test	Histology	n(%)
-	-	165(77.8)
-	+	10(4.7)
+	-	8(3.8)
+	+	29(13.7)

B

CLO-test	Histology	H. pylori antigens	n(%)
-	-	+	15(9.0)
+	-	+	5(3.0)
+	+	+	137(82.5)
+	+	-	2(1.2)
-	+	+	7(4.2)

Table 3: The strains other than *H.pylori* cultured from the gastric mucosa subjected to *H. pylori* antigens testing; in all cases the results were negative.

Kingella sp.
Moraxella sp.
Eikenella corrodens
Haemophilus parainfluenzae
Pseudomonas aeruginosa
Acinetobacter sp.
Rothia dentocariosa
Rothia mucilaginosa
Streptococcus salivarius
Proteus vulgaris
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus hominis
Staphylococcus xylosus
Raoultella sp.
Budvicia aquatic
Enterobacter gergoviae
Klebsiella pneumonia
Hafnia alvei
Yersinia kristensenii

Table 4: The cultured standard strains which were subjected to *H. pylori* antigens testing; a positive result was obtained only for *H. pylori* ATCC 43504.

Strain
Escherichia coli ATCC 25922
Proteus mirabilis ATCC 29245
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 29213
Staphylococcus epidermidis ATCC 12228
Staphylococcus xylosus ATCC 29971
Streptococcus salivarius ATCC 13419
Helicobacter pylori ATCC 43504

lonal anti-*H. pylori* antibodies combined with horseradish peroxidase were added. After an incubation of 60 minutes, unbound antibodies were washed out, and tetramethylbenzidine, a substrate for horseradish peroxidase, was added to the remnants in the plate microwells. The peroxidase reaction was stopped by adding sulphuric acid. The intensity of the yellow colour obtained was assessed by means of spectrophotometry at 450 nm.

Results

Bacteria were cultured from endoscopic specimens of gastric mucosa for 378 patients. A positive result of the culture was obtained in 166 (43.9%) patients, and their bacterial colonies were assessed for the presence of *H. pylori* antigens. A positive result was obtained in 164 patients (98.8%). In patients whose growth of bacteria other than *H. pylori* was obtained from endoscopic specimens and whose assessment for the presence of *H. pylori* antigens was performed, no positive results were obtained for any such cases (Table 3). A negative result in the test for the presence of *H. pylori* antigens was also obtained in tests for reference bacteria strain cultures – *Staphylococcus aureus, Staphylococcus xylosus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Streptococcus salivarius*, while a positive result was obtained for a *H. pylori* reference strain culture (Table 4).

In 15 patients (9.0%) with a positive culture confirmed by the presence of *H. pylori* antigens, both the urease test (CLO test) and the result of the histological examination were negative (Table 2). In 12 other patients (7.2%) with a positive culture also confirmed by the presence of *H. pylori* antigens, only one of the two tests (CLO test or histological test) was positive. In the remaining 137 patients (82.5%) with a positive culture and a positive test for the presence of *H. pylori* antigens, both the results of the CLO test and the histological examination of endoscopic specimens were positive. A positive result of CLO test and histological examination was also obtained in 2 patients for whom the culture was positive, but this result was not confirmed by the test for the presence of *H. pylori* antigens; *Pseudomonas aeruginosa* was identified in both cases.

Discussion

The identification of *H. pylori* bacteria from a culture using the classical method is not accepted by some researchers [3]. Their doubts are justified by the fact that some bacteria may have the morphology of colonies similar to *H. pylori*, they are Gram-negative just like *H. pylori* and they are characterized by urease, catalase and oxidase activity identical with *H. pylori*. The results of the present study showed that a discrepancy between the diagnosis based on the classical criteria and the result of a test for the presence of *H. pylori* antigens occurred only in two cases in a relatively large group of patients. In both cases, *P. aeruginosa* was the bacterium yielding a positive result in the classical method used for the identification of the *H. pylori* bacteria.

Preparing a microscopic glass from a bacterial culture and the assessment of *H. pylori* index enzymes takes less time than performing an immunological test assessing the presence of bacterial antigens. The cost of the assessment of *H. pylori* antigens is several times higher than the cost of *H. pylori* identification in the classical method. However, the advantage of the immunological test over the classical method for *H. pylori* identification from culture relies on a lower number of false positive results.

The use of proton-pump inhibitors was not an exclusion criterion for the study, and this is probably the source of discrepancy between the results of CLO test, histology, and the culture. The currently obtained results are consistent with data presented by other authors and with our own published previously [5, 6, 13, 14, 17].

Approximately 500 various strains of bacteria are present in the gastrointestinal tract [18], and the number of them are in the stomach [17]. Thirty bacteria occurring in faeces were tested by the manufacturer of the immunological test for detection of *H. pylori* antigens (the result of this analysis was presented in the leaflet enclosed with the commercial kit); none of the bacteria tested by the manufacturer gave a positive result for the presence of *H. pylori* antigens. Our own assessment of the bacteria other than *H. pylori* grown from endoscopic specimens of the gastric mucosa proves that these bacteria did not give a positive result of *H. pylori* antigens. For 3 bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*), our result corresponded to the result presented in the manufacturer's leaflet.

Conclusions

The classical method for *H. pylori* identification from culture is characterized by high accuracy. Therefore, it is not

necessary to extend the diagnostic criteria to molecular research or an assessment of the presence of *H. pylori* antigens. Nevertheless, a positive result of the test for the presence of *H. pylori* antigens from the culture could be the additional evidence which confirms the culture result in selected cases.

Acknowledgements

The study was supported by Medical University of Bialystok, Grant No. 113-18667

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Received: 2017
Accepted: 2017