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A LOW COST METHOD TO PRODUCE A GASEOUS ENVIRONMENT FOR THE ISOLATION OF Helicobacter pylori

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SUMMARY

A low cost method (LCM) to produce a gaseous environment for the isolation of **Helicobacter pylori**, was compared with the standard Gas Park system. The LCM uses a carbonated antacid tablet, a plastic bag with tap water, a candle, and a wide-mouthed glass jar provided with a tight-fitting metalic screw cap and a rubber gasket. Antral gastric biopsies from 153 cases were incubated by duplicate on blood agar plates and treated with the two methods. In 95 cases the agent was isolated from both, and only from the standard method in 10 cases; the opposite condition was found in five cases, and 43 were negative. That difference is not significant (Pearson's $X^2 = 93.25$ $p > 0,05$).

KEY WORDS: Helicobacter pylori: Technical assay; Microaerobiosis.

INTRODUCTION

Helicobacter pylori is a microaerophilic bacteria isolated in 1982 from gastric mucosa [1,2]; it was previously called **Campylobacter pyloridis**[4] and then **C. pylori** [5]. At present it is the most important infectious agent associated with gastritis type B and peptic ulcers [6,9].

The microaerophilic atmosphere required for its growth could be produced in an anaerobic jar without catalyst, using a disposable H_2, CO_2 generator envelope, such as Gas-Pak (BBL); or with other methods as the evacuation-replacement system[7]. These systems are practical but expensive and not available in all laboratories, specially those in developping countries. For this reason we tested an alternative and more economical method, inspired by that described by PENNIE et al. for **Campylobacter jejuni** cultivation[8].

MATERIALS AND METHODS

For the method proposed here, a gallon glass jar with a wide mouth and a tight-fitting metalic screw cap (with rubber gasket) was used. The reducer and CO_2 generator system a lighted candle and a carbonated effervescent antacid tablet (Alka-Seltzer [R]). This system was compared with the standard method, which consists of an anaerobic jar without catalyst and a CO_2, H_2 generator Gas Pak envelope (BBL Microbiology Systems, Cockeysville, MD 21030).

Both methods produce a microaerophilic environment and were used for the isolation of **H. pylori** from gastric antral biopsies. These were obtained from 143 consecutive patients submited to gastroendoscopy and ten volunteers. At least two antral biopsies were obtained from each case; one was used for bacteriological isolation and the other(s) for histology. The former was rubbed across the surface of two fresh blood agar plates[3] that were then incubated at 37 °C for five days; the first one of them using the standard method and the other by the low cost method (LCM). Isolated bacteria were identified as **Helicobacter pylori** on the basis of their typical colonial morphology, Gram stain, oxidase, catalase and urease reactions.

Additionally, seven decimal-dilutions of suspensions adjusted by the McFarlane turbidimetric method to approximately 10^8 CFU/ml from six recently isolated strains of **H. pylori** were tested. Each dilution was inoculated by triplicate, evenly on two separate sets of blood agar plates and incubated at 37°C in each of the gaseous environments as was described.

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RESULTS

Both methods produced similar results for 95 (62%) cases in which **H. pylori** was isolated, and from 43 (28%) culture-negative samples. However, 15 cases were culture positive in only one of the systems: ten in the standard and five in the LCM (Table 1).

The differences between the number of isolations with either of the systems are not significant (Pearson's $X^2 = 93.25$, $p > 0.05$). In additon, the suspensions of **H. pylori** grew well in both gaseous environments tested; but, the number of colonies in each plate was more than 300, and no significant difference was observed. Also, the size of the colonies was similar in both conditions; however, independently of the atmosphere used, in some plates the diameter of colonies obtained were smaller (**ca** 0.5 mm) than the normally seen.

Table 1
Comparison between isolation of **Helicobacter pylori** using a H_2, CO_2 generator envelope and a low cost system

Low cost syst.	H_2, CO_2 generator envelope		
	Nº of positives	Nº of negatives	Total
Nº of positives	95	5	100
Nº of negatives	10	43	53
Total	105	48	153

$X^2 = 93.25$ $p > 0.05$

DISCUSSION

The microaerophilic environment required for the growth of **H. pylori** can be obtained by both methods tested. In the LCM the reducer system is performed by a lighted candle and a carbonated effervescent antacid tablet. The candle reduce the oxygen level to approximately 17 to 19%[7], that is then diluted by the increased level of CO^2, generated by the carbonated effervescent antacid tablet. The final gas environment obtained permits the isolation of **H. pylori** and its cost is less than 0.1 USA Dollar. Also, the cost of the system can be reduced more using a common wide-mounted glass jar insted the anaerobic jar.

Both methods tested yield the same results in 90.2% of the analized samples, and incongruous data was obtained from 15 cases. Nevertheless, that difference is not significant ($p > 0.05$). Then, it could be also possible that the agent was isolated only in one system from those patients with light infections, because in all of those cases, there were less than ten colonies per plate, including three with only two colonies. Also, no significant differences were found when suspensions of this agent were incubated in each one of the gaseous environments tested.

These findings indicate that the economical system proposed is suitable for the isolation of **H. pylori** from antral biopsies and for its subsequent culturing.

RESUMEN

Un método de bajo costo para producir el ambiente gaseoso para el aislamiento de *Helicobacter pylori*.

Se comparó un método de bajo costo (MBC) para producir el ambiente gaseoso para el aislamiento de **Helicobacter pylori**, con el sistema estándar de gas Pak. El MBC usa una tableta carbonata de antiácido, una bolsa plástica con agua, una candela y un frasco de vidrio con boca ancha, provisto de tapa metálica de rosca con empaque de hule. Las biopsias de antro de 153 pacientes se inocularon por duplicado en platos de agar sangre y se incubaron bajo los dos sistemas. En 195 casos el agente se aisló de ambos platos, y sólo del incubado bajo el método estándar en diez casos; la condición opuesta se presentó en cinco casos; 43 casos fueron negativos. Esa diferencia no es significativa (X^2 de Pearson = 93,25 $p > 0,05$).

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