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A SIMPLE AND INEXPENSIVE METHOD TO GENERATE A MICROAEROPHILIC ATMOSPHERE FOR THE ISOLATION OF *CAMPYLOBACTER* SP.

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SUMMARY

Faeces of 138 chickens were inoculated on Blaser agar plates. One set of plates was incubated in jars with CampyPak envelopes. The others were incubated in "Zip-lock" plastic bags (7 x 8 in.) and a microaerophilic atmosphere was generated exhaling into the "Zip-lock" plastic bag, after holding the breath for 20 sec. Then, the bag was pressed to evacuate its atmosphere, inflated again, and pressed (4 times), and finally sealed. *Campylobacter* was isolated from 127 (96.2%) of samples incubated in jars with gas generator envelopes and from 129 (98%) of the specimens incubated into the bags. The proposed methodology offers good savings for cost-conscious laboratories.

KEYWORDS: *Campylobacter:* technical assay; Microaerobiosis, poultry.

INTRODUCTION

Campylobacter is acknowledged as the leading bacterial cause of diarrhea in the United States of America, causing over 2 million cases annually[8]. A similar situation occurs in other developed countries; for example, the annual estimated cases in The Netherlands are 300,000[7]. In developed countries the human campylobacteriosis are usually associated with contaminated food. Undercooked poultry products are probable the main source of infections, at least for sporadic cases[3], because *Campylobacter* species are inhabitants of the intestinal tracts of poultry[2, 3]. Over 80% of chickens analized in different countries showed these bacteria in their faeces[5].

Other important sources of infection, that may be are more important in developing countries, are untreated drinking water, raw milk, and vegetables irrigated with sewage, which was not adequately treated[1]. For that reason it is possible that in developing countries this agent plays a more important role in the causality of human diarrhea. The lack of data from the majority of these areas are maybe owing to the hight cost of the isolation methods for this agent.

The isolation of *Campylobacter* is expensive due to the culture media and the microaerophilic atmosphere required (85% N_2, 10% CO_2, and 5% O_2). This atmosphere is achieved with CampyPack or other similar systems. They are relatively expensive and unwieldy for routine use, at least in developing countries (these envelopes cost **ca.** USA $ 1.5 and a jar cost **ca.** $ 450). However, some cheaper alternatives have been described, one of which utilizes a plastic bag insted of a jar, and from which the atmosphere is evacuated and replaced with a commercially purchased gas mixture (Campy Bio-Bag Cfj System, Marion Scientific). The most inexpensive method was proposed by KAPLAN et al.[6]: the operator holds his breath for 10 seconds and then exhales into the plastic bag; however, this method fails to recover about 15% of all isolates. The aim of this paper is to propose a combination of the last two methods, resulting in a higher isolation rate.

MATERIAL AND METHODS

Fecal samples from 138 chickens randomly chosen from a butcher plant (Costa Rica) were inoculated by duplicate on Blaser agar plates (12 to 24 chickens by week). A set of plates was incubated in a jar and the microaerophilic atmosphere was generated with the CampyPak system (12 plates by jar). The other plates were incubated in "Zip-lock" plastic bags (7 x 8 in.) and the microaerophilic atmosphere was generated holding the breath (**ca.** 20 sec) and exhaling into the bag through a rubber-hose (0.5 cm diameter by 80 cm long) rolled in loopes

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(**ca**. 10 cm diameter). The bag was then pressed to evacuate its atmosphere and the procedure (exhaling and pressing) was repeated 4 more times and the inflated bag was sealed rapidly. Both sets of plates were incubated 48 hrs at 42°C. Isolates were identified by Gram stain, oxidase, and catalase. Twelve of the isolates strains were biochemically identified using the Api-campy system. Four strains, identified as *C. jejuni jejuni*, were ten fold diluted and inoculated by duplicate on blood agar plates. One set was incubated in a jar and the other in a plastic bag, as described.

RESULTS

Campylobacter was isolated from 127 (96.2%) out of 138 samples incubated in jars with gas generator envelopes and from 129 (98%) of the same specimens incubated in the plastic bag. The 12 strains identified biochemically were *C. jejuni jejuni*. Moreover, the number of colonies from the ten fold dilutions of the 4 strains tested were similar in both atmospheres.

DISCUSSION

If an economical method is available for the isolation of fastidious agents, such as the case of *Campylobacter*, the information of these agents from developing countries may become more abundant, and their role in infectious diseases will be better understood. Previously, another method was evaluated, in that, the microaerophilic atmosphere was generated in a gallon glass jar using a lighted candle and a carbonated effervescent antiacid tablet (AlkaSeltzer^{R}), that was very efficient in the isolation of *Helicobacter pylori*, but not *Campylobacter*[4].

The proposed methodology combines evacuation and replacement with the method of KAPLAN et al.[6], and allows the isolation of *Campylobacter* without the use of gas-generator envelopes, showing a similar or higher isolation rate, compared with the jar and gas-generator envelope method. The size of the bag (7 x 8 in.) is important, because in bigger bags is difficult to get the appropriate microaerophilic environment by the method described and the isolation rate is lower. This low cost method was very efficient and offers good savings for cost-conscious laboratories.

RESUMEN

Un método simple y económico para generar la atmósfera microaerofílica para el aislamiento de *Campylobacter* sp.

Se inocularon por duplicado 138 muestras de heces de pollos en agar de Blaser. Un juego de las placas se incubó en jarras con sobres generadores de microaerobiosis (CampyPak). Las otras placas se incubaron en bolsas plásticas tipo "Zip-lock" (7 x 8 pulgadas) y la atmósfera microaerofilica se generó inflando la bolsa luego de retener la respiración por 20 seg, desinflándola e inflándola nuevamente (4 veces) y finalmente sellándola. Se aisló *Campylobacter* de 127 (96.2%) de las muestras incubadas en la jarra y de 129 (98%) de los especimenes incubados en la bolsa. La metodologia propuesta ofrece una posibilidad económica para los investigadores concientes de los costos de operación.

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