INTRODUCTION

Campylobacter is a leading cause of gastroenteritis around the world. Symptoms include severe diarrhoea, vomiting, and fever. Infection is normally self-limiting in previously healthy adults, lasting 3-5 days. However, Campylobacter has been reported to cause systemic infection, linked to the development of Gullain-Barre syndrome. The most common route of human infection is through ingestion of undercooked, contaminated poultry. Campylobacter can be isolated from the stool of infected individuals in microaerobic atmosphere (5-10% O2, 5-10% CO2, and 85% N). There are currently a number of different methods available to achieve microaerobic conditions with very little evidence in the literature of a gold standard technique. This suggests a requirement for investigation into the different techniques available, to ensure continuity and quality of isolation methods in diagnostic laboratories.

METHODS

Isolation Rate Analysis

Stool samples (n=466) that arrived at the laboratory were given a unique identification number. 1-2ml of each sample was inoculated in triplicate onto Preston agar as per routine protocol. This selective medium repressed the growth of the normal intestinal microflora. Both the MACS cabinet and the jars contained a sub-cultured Campylobacter control and a negative control of E. coli ATCC 25922 on Preston agar.

MACS Cabinet

One of the triplicate plates was incubated at 42°C for 48 hours in a MACS cabinet. The cabinet was supplied with a commercial anaerobic gas mixture which was combined with air to create optimal atmosphere (figure 1).

CampyGen

The second plate from the same sample was placed in an anaerobic jar and 3 CampyGen gas generating sachets were placed inside the jar before it was sealed. Microaerophilic atmosphere is reached through partial consumption of O2 and CO2 generation by the sachets.

Gas Replacement System

The third plate from the same sample was placed in an anaerobic jar. Using the gas replacement system, half the air from the jar was vacuumed and replaced with anaerobic gas (AnaeroPack) to reach the desired gas composition. The jar was placed in an air incubator at 42°C for 48 hours.

Microscopic Identification

The plates were examined in parallel for the presence of Campylobacter colonies. Any suspect colonies were used to prepare Gram films for confirmation. Campylobacter has a characteristic gull-wing appearance on microscopy and is therefore distinguishable from other Gram negative bacilli.

Quantifying Bacterial Growth in Each Method to Compare Efficacy

Serial dilutions of 10⁻¹, 10⁻², and 10⁻³ of Campylobacter were plated in triplicate and incubated in all 3 of the above methods. The average number of colonies was counted from each plate to identify the method that reproduced the greatest volume of growth.

RESULTS & DISCUSSION

Isolation Rate Analysis

Plates incubated in the MACS cabinet, CampyGen, and gassing system jars yielded an isolation rate of 2.7%, 2.5% and 3.7%, respectively (figure 1). 100% of all positive samples were observed in the gassing system jars.

The average number of colonies cultured was comparable across all 3 of the techniques. Incubation in the MACS cabinet yielded discrete bead-like colonies, measuring 0.5-1.0 mm in diameter (A). However, incubation in both the CampyGen and gassing system jars yielded large spreading colonies, measuring 1.0-10 mm in diameter. The literature states that this may be due to increased humidity in the jars.

Colonies cultured in the jars usually displayed confluent growth (B)(figure 4).

Cost Comparison of Current Campylobacter Incubation Method and Proposed Methods

Changing from the MACS cabinet method to the gas replacement system would save the department £4,727.59 per annum, reducing costs by 99.6%.

CONCLUSION & FUTURE WORK

Adapting the current protocol to the gas replacement technique would not only reduce costs for the department, but also increase the isolation rate of Campylobacter from patient stool samples. It is important to identify Campylobacteriosis to ensure the patient receives the correct treatment particularly in cases where patients are immunocompromised. Campylobacter infection is also a reportable disease, meaning Public Health are alerted when the organism is isolated from patient samples. This allows the source and any contacts of infection to be traced, aiding the effort to reduce contamination of food products and protect public health. As a result of this investigation, the Medical Microbiology department at Aberdeen Royal Infirmary have changed their protocol for Campylobacter isolation to adopt the gas replacement method.

The International Organization for Standardization published a document that states optimal atmospheric conditions for Campylobacter culture is 5-10% O2, 5-10% CO2, and 85% N. However, it is possible that variation in the exact composition of gas used exists in diagnostic laboratories around the world due to differences in techniques. There is currently no research in the literature into the effects of different gas compositions on the culture of Campylobacter. Therefore, it may be possible from future study to identify a closer range of optimal conditions and the effects different atmospheric conditions has on recovery of the organism.

There are many different selective media for the isolation of Campylobacter, including Preston, Campycolsel, and CCDA. Interestingly, there is little evidence in the literature regarding the effects of using different selective media on the culture of Campylobacter. However, there is evidence in the literature of the effects of different media on species level identification using MALDI-TOF. Therefore, further investigation into these effects may aid diagnostic laboratories in improving the isolation rate of the organism from patient samples.

Key References