SELECTIVE MEDIA FOR *CAMPYLOBACTER*

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RESEARCH ARTICLE

Abstract

They require special cultivation conditions: microaerophilic environments, enriched media, and a growth temperature of 42-43ºC, with an incubation period of 48-72 hours. Acute infection leads to short-lived immunity. Patients exhibit specific IgG, IgM, and IgA in their serum, with IgA also present in intestinal secretions. The pathogenic capacity of C. jejuni varies from strain to strain and is determined by multiple factors related to the bacterium or the host. Factors influencing virulence characteristics include toxin production, cell surface structure, and iron utilization in the bacterium's metabolism. C. jejuni produces enterotoxin, cytotoxin, and endotoxin.

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INTRODUCTION

Campylobacter bacteria are commensals in the intestines of animals, primarily birds such as chickens, turkeys, ducks, seagulls, and also in mammals like cattle, sheep, dogs, and cats. These bacteria contaminate milk, food, and drinking water where they can survive for several weeks.

The survival of these bacteria in food depends on the type of food and its storage conditions. For example, in raw cow's milk, Campylobacter can survive for three weeks at 4°C and three days at 25°C, but it dies within a minute at 60°C. In cheese, they do not grow due to the acidity and salt content.

In certain meats, such as poultry carcasses, which are often heavily contaminated, these bacteria can multiply when stored at high temperatures of 35-42°C. Furthermore, the bacteria can survive for several months or weeks in refrigerated or frozen meat. Their survival is longer in vacuum-sealed red meat.

Campylobacter bacteria are sensitive to dry conditions and room temperature. C. jejuni is sensitive to various disinfectants such as sodium hypochlorite, phenol, ethyl alcohol, glutaraldehyde, and monochloramine. Therefore, chlorination of water has found practical application in preventing these infections. They are sensitive to erythromycin, macrolides, gentamicin, chloramphenicol, and tetracycline. They are resistant to trimethoprim and vancomycin.

C. jejuni strains are antigenically diverse, with at least 42 serotypes distinguished based on thermostable O antigens. Additionally, flagellar proteins are also antigenic in nature and can be used in serotyping.

MATERIAL AND METHODS

I conducted a prospective study based on microbiological diagnoses recorded in the bacteriological registry of the S.C. Diaser medical analysis laboratory in Oradea. To carry out the study, archival data from the laboratory's specific computer program were also consulted, as well as the computerized data base of the unit.

Materials required for the examination:

- One collection container (feces collector with a collecting spoon) with a transport medium
- Wooden spatulas
- Latex gloves

For stool culture, a 5-10g sample of fecal matter should be collected and placed in the feces collector with a transport medium. If the stool is liquid, 5ml should be collected. It is recommended to choose a liquid, mucous, or bloody portion, if available. Do not collect quantitiess greater than 10g, as this may

reduce the chances of isolating pathogenic bacteria.

Isolation of aerobic bacteria.

• The sample is inoculated onto two culture media, one with weak selectivity (Mac Conkey) and one with moderate selectivity (Hektoen), and incubated for 24 hours at 35-37°C. Cultures are observed at 24 and 48 hours for the appearance of characteristic colonies. For the Vibrio genus, the recommended selective medium is BSA (bile salts agar), and for yeasts, Sabouraud agar with Chloramphenicol is used.

• To increase the chances of isolation, the sample is subcultured on enrichment media that promotes the multiplication of pathogens (selenite broth for Salmonella spp., alkaline peptone water or taurocholate peptone broth at pH=8.0-9.0 for Vibrio). After incubation for 24 hours at 35-37°C, smears and cultures can be performed from the upper part of the medium. Subsequent transfers are made to culture media.

RESULTS AND DISCUSSIONS

The abundant inoculation with fecal material suspension is performed, followed by a 24-hour incubation at 42°C. Subsequently, the transfer is made to selective media, preferably Preston selective agar for Campylobacter.

The range of selective media for Campylobacter was significantly expanded in 1977 when Skirow established the requirements for gaseous incubation, essential nutritional factors, and the inclusion of antibiotics and inhibitors for associated flora.

Table 1 presents a series of selective media specifically recommended for isolating Campylobacter jejuni and C. coli species from fecal material and food samples with a rich associated flora.

Table 1

Selective Media for *Campylobacter*

The medium	Nutrient component	Selective component	The aspect of colonies
Skirow	Columbia blood agar with horse blood."	Vancomicina Trimetroptim Polimixină	1 mm semi-transparent round, bulging wet surface tendency to spread on streaks
Butzler	Columbia agar with defibrinated horse blood.	Novobiocină Trimetoprim Polimixină	1-2 mm semitransparente, shiny metallic grey, non-hemolytic round.

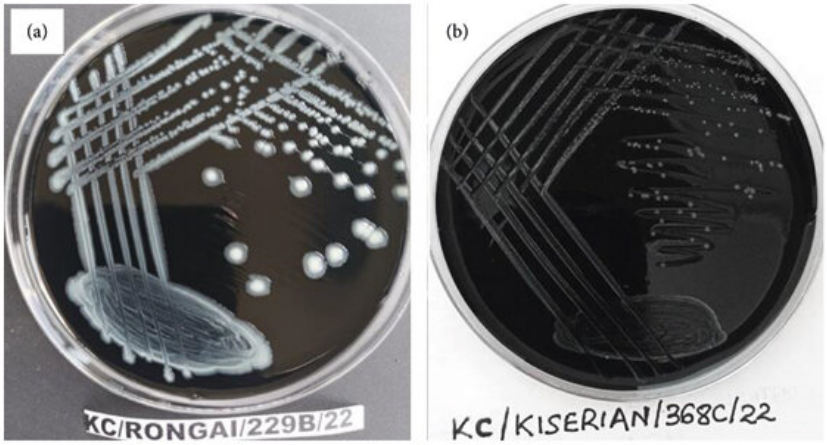

Fig. 1. **Campylobacter colonies on mCCDA plate after 48 hours of microaerobic incubation at 42°C. Medium off-white glistening/shiny and spreading colonies (plate (a)) and the small gray colonies on the media (plate (b)).** https://www.researchgate.net/figure/Campylobacter-colonies-on-mCCDA-plate-after-48-hours-of-microaerobic-incubation-at-42C_fig2_363861469

The data presented in the "Prevalence Studies of Campylobacter spp. in Chicken Farms" in the Veneto region, conducted by Antonia Ricci, Serena Amato, and Lisa Barco as part of the food safety plan in the Veneto region (2002-2004), which aimed to activate surveillance and monitoring systems capable of providing reliable information regarding the health status of farms and the level of food contamination, served as the cornerstone of control activities.

The monitoring activity made it possible to highlight the high prevalence of Campylobacter in broiler chickens at the time of slaughter. These data confirm the need to implement rigorous biosecurity measures to prevent the introduction of the pathogen into the farm. In fact, it is widely demonstrated that when the microorganism from the environment or other external sources manages to colonize an animal, it spreads rapidly among all members of the group, reaching prevalence rates close to 100% at the time of slaughter.

Nearly all strains isolated from chickens are attributed to the species Coli and Jejuni, which are the agents primarily responsible for human campylobacteriosis episodes. Additionally, it has been possible to determine how often different Campylobacter species coexist within different groups among those examined.

Extremely high prevalences were also found in carcasses sampled at the end of the slaughter operations. However, it should be noted that the carcasses sampled were taken before being subjected to refrigeration or freezing, treatments that appear to result in a significant reduction in meat contamination. Therefore, the data obtained are likely to overestimate the actual contamination of poultry meat in the market. On the other hand, it should not be forgotten that Campylobacter is a microorganism characterized by extremely low infectious doses, and thus, a few hundred bacterial cells may be sufficient to cause food poisoning.

CONCLUSIONS

1. Isolation can be achieved through enrichment via membrane filtration.
2. Selective media isolation (Skirrow, Campy agar) is carried out from fecal samples and food specimens.
3. Incubation is conducted at 42-43ºC for 48-72 hours under microaerophilic conditions.
4. Identification is based on colonies resembling honey drop shapes that

have grown under microaerophilic conditions.

5. At temperatures of 42-43ºC, microscopic examination of the colony, oxidase-positive characteristics, and other biochemical traits, such as the absence of urease production and the lack of H2S production on TSI medium, differentiate C. jejuni from C. coli.
6. Enrichment in liquid media with suitable inhibitors is important for isolating C. jejuni from foods, while direct dispersion onto appropriate selective media is preferred for fecal specimens.
7. In recent years, new emerging agents have been identified, and the number of associated pathologies has increased. Among the causes of this rise, we can include demographic and behavioral changes, such as an increased susceptibility rate, the consumption of easily contaminated foods, and the consumption of food products in mass catering, as well as the widespread diffusion of foods derived from industrialized processes.

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