PostScript

LETTERS TO JCP

Efficient isolation of campylobacters from stools: what are we missing?

The past three decades have witnessed the rise of campylobacter enteritis in humans from virtual obscurity to notoriety, with present isolation rates superseding those of other enteric pathogens such as *Salmonella* spp. and *Shigella* spp. in most developed countries. Unlike the salmonellae and other enteric pathogens, most (~ 99%) clinical reports concerning campylobacter are sporadic, and campylobacter enteritis outbreaks are rare. Although campylobacters are not completely new to applied bacteriology, they have largely evaded traditional techniques used for the isolation of pure cultures, apart from single isolations that were free from competing organisms. Until the development of a selective medium by Skirrow,[1 2] these organisms were known mainly by veterinarians as animal pathogens, which were responsible for a wide variety of disorders in cattle, sheep, and pigs.[3] Since the development of more sophisticated isolation techniques, the true disease potential of these organisms has become apparent and today campylobacteriosis is regarded as a zoonosis, which is capable of being transmitted to humans by a wide range of domestic animals.[3]

There have been several reports describing the inability of selective media to recover certain species of campylobacter, especially the catalase weak or negative organisms, from faecal specimens.[4 5] In addition, there are large epidemiological differences between rates of infection with campylobacters between Northern Ireland and the rest of the UK; however, no data exist with regard to the rates of isolation of the atypical campylobacters from stool specimens locally. Therefore, it was the aim of our study to evaluate the efficacy of recovery of clinically relevant campylobacters from faecal specimens in Northern Ireland, using direct plating and differential filtration techniques.

One hundred and eighty six faecal specimens from an equal number of patients with acute gastroenteritis were examined for the presence of *Campylobacter* spp. over the peak seasonal period, May to June. Faecal specimens were obtained from family practitioners in the community and were examined within 24 hours of receipt. Two isolation methods were compared for their efficacy in recovering viable organisms, namely: (1) direct plating of faeces on to two selective media at two different incubation temperatures (37°C and 42°C); and (2) differential filtration on non-selective medium incubated at 37°C. For both treatments, 0.5 g faeces was emulsified in 0.1% (wt/vol) peptone water. For direct plating, 10 µl of faecal suspension was inoculated on to both modified CCDA agar (Oxoid Ltd, Poole, Dorset, UK), containing cefoperazone (32 mg/litre) and amphotericin B (10 mg/litre), which was subsequently incubated at 37°C, and also on to Preston's selective medium (Oxoid Ltd), containing rifampicin (10 mg/litre), trimethoprim (10 mg/litre), cycloheximide (100 mg/litre), and polymyxin B (5000 IU/litre), which was incubated at 42°C. For recovery by differential filtration, 300 µl of faecal suspension was passively filtered through a 0.65 µm cellulose triacetate membrane (Millipore, Edinburgh, UK), and incubated at 37°C, as described previously.[6] In both treatments, plates were incubated in microaerophilic conditions (5% (vol/vol) O_2) for two to five days. Presumptive positive colonies were further characterised as described previously.[7]

By direct plating, 18 of 186 (9.7%) faecal specimens were positive, whereas 22 of 186 (11.8%) specimens were positive by the differential filtration method using non-selective media (table 1). All campylobacters that grew on one or other selective medium were also isolated by differential filtration, except for *Helicobacter fennelliae*, which was only isolated by non-selective filtration. The use of both media together missed thermophilic campylobacter in three specimens, which were positive by filtration. Statistical analysis was performed using a paired Student's *t* test and this gave a probability of $p = 0.0226$, demonstrating a significant difference between differential filtration and selective plating techniques.

Overall, our study shows that non-thermophilic campylobacters were not commonly isolated from faeces and that the use of a combination of selective media was superior to the use of one selective medium only, and that use of differential filtration with a non-selective medium was superior to direct plating on selective agars. Surprisingly, only one atypical organism, *H fennelliae*, was isolated from 186 patients, and direct plating failed to detect up to six strains of *Campylobacter jejuni*. Similar to our study, in England and Wales, the infectious intestinal diseases study[8] noted remarkably few cases of other organisms, including *Campylobacter upsaliensis*, *Campylobacter fetus*, *Campylobacter hyointestinalis*, and *Campylobacter lanienae*,[9] whenever a filtration method was used. However, this study did not comment on the number of *C jejuni* strains missed by selective plating, which was significant in the Northern Ireland study. Given that approximately 1000 laboratory reports for campylobacters from faeces in Northern Ireland are currently received by the Communicable Disease Surveillance Centre (Northern Ireland) annually, extrapolation of recovery rates based on our study would equate to approximately 27 cases being undetected in the laboratory.

Most clinical laboratories in the UK isolate campylobacters from stools at incubation temperatures of greater than 40°C, usually 42–43°C. In our study, we noted a slightly higher isolation rate for Preston's selective agar incubated at 42°C, compared with the CCDA medium, which was incubated at 37°C, where an additional three specimens were positive using the former technique. Previously Bolton *et al* noted that a higher recovery rate was made from CCDA medium at 37°C as opposed to 42°C.[10] Although the Public Health Laboratory Service standard operating procedure for the investigation of faeces for the presence of campylobacters[11] recommends an incubation temperature of 35–37°C for primary isolation, perhaps the use of a temperature of greater than 40°C would be better.

Moore and Murphy[12] previously demonstrated that the use of selective agents in laboratory media may result in the failure to recover sensitive strains. In our present study, the inability of the selective media used to recover all strains might result from the sensitivity of these wild-type *C jejuni* and *H fennelliae* to the antibiotics incorporated within the selective formulations.

Presently, in Northern Ireland, most clinically relevant campylobacters from faeces are being isolated on either Preston's or Skirrow's

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Table 1 Comparison of recovery of campylobacters from human stools using selective plating versus non-selective differential filtration techniques

Isolation technique (incubation temperature)	Total no. faeces examined	Number of specimens positive (% positive)	Species identified
Differential filtration (37°C)	186	22 (11.8%)	21 *Campylobacter jejuni* 1 *Helicobacter fennelliae*
CCDA selective agar (37°C)	186	15 (8.1%)	All *C jejuni*
Preston's selective agar (42°C)	186	18 (9.7%)	All *C jejuni*

selective agar. Two laboratories are using Preston's medium, six laboratories are using Skirrow's medium, an additional laboratory is using selective enrichment with Preston's medium, and no laboratories are routinely using filtration. Hence, this may result in the under-reporting of approximately 3% of antibiotic sensitive *C jejuni* isolates, in addition to the non-thermophilic campylobacters. Although the non-thermophilic campylobacters are not an important cause of diarrhoeal disease in the UK, their true prevalence may have been under-reported owing to the use of techniques that were unsuitable for their optimal isolation from human stools.

Overall, the use of an additional selective medium and filtration may improve the recovery rate of campylobacters from faecal specimens. However, the adoption of such additional protocols has important implications for both the management and resources of routine faecal microbiology. Because it may not be cost effective to introduce double media and/or filtration protocols as part of the routine diagnostic investigation of faecal specimens for campylobacters, the use of total pathogen screening using the multiplex polymerase chain reaction may prove a sensitive and specific alternative, where only positive stools are subsequently cultured, and using extended culture techniques when indicated.

Therefore, we conclude that differential filtration of faecal specimens for the detection of campylobacters should be included as an additional algorithm following negative results by direct plating, particularly in AIDS/human immunodeficiency virus positive patients, in patients with haematological malignancies, in patients with cancer undergoing immunosuppressive chemotherapy, and in populations where atypical campylobacter strains might be of epidemiological importance, including homosexual men. In addition, we would advocate the use of this technique on stool specimens from patients thought to be involved in outbreaks from whom no other pathogen has been isolated.

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Evidence for antibiotic induced *Clostridium perfringens* diarrhoea

We read with interest the paper entitled "Evidence for antibiotic induced *Clostridium perfringens* diarrhoea".[1] The authors review the current knowledge of this syndrome and discuss the need for routine screening. To contribute to this debate we present the results of a recent survey performed in our laboratory. *Clostridium difficile* is a major cause of antibiotic associated diarrhoea (AAD) but an increasing number of reports implicate *C perfringens* as a cause of this condition.[2] Because our hospital has a substantial number of cases of AAD, we decided to perform a survey of the incidence of *C perfringens* enterotoxin in stools from hospital inpatients.

Over a six month period, all inpatients who presented with loose or watery stools submitted to the microbiology department were included in our study. Anyone who had tested positive for *C difficile* or *C perfringens* within the previous six months or anyone who had already been enrolled as a case was excluded, so that only incident cases were investigated. For all cases, a detailed questionnaire was completed to try to ascertain the risk factors predisposing to AAD caused by either of these two pathogens.

Each stool sample was tested for *C difficile* toxins A and B in addition to *C perfringens* enterotoxin using commercially available enzyme linked immunosorbent assay kits (TechLab, Blacksburg, USA). All samples were also processed for other bacterial pathogens using standard methods. Of the 249 samples tested, 24 (9.6%) were positive for *C difficile* enterotoxin; however, only four (1.6%) were positive for *C perfringens* toxin. None of the samples was positive for both *C difficile* and *C perfringens* toxins and no other bacterial pathogens were isolated.

Of the 24 *C difficile* positive patients, 18 were over 60 years of age. Half of the positive patients had clinical diarrhoea (more than three loose stools each day). Eighteen of the 24 had received previous antibiotic treatment, with flucloxacillin and cefuroxime being the most frequently used, either singly or in combination with other antibiotics. Only one patient had received clindamycin. The presence of severe or disabling underlying disease was reported in 17 of the positive patients. Five positive patients received antibiotic treatment with metronidazole .

All of the four patients with *C perfringens* toxin were women, their respective ages were 55, 72, 92, and 94. Two were in medical wards and the other two were from renal wards. Only one was recorded to have clinical diarrhoea. Of the positive patients, three had disabling disease and only one had antibiotic treatment before developing diarrhoea. None of the positive patients required antibiotic treatment.

Clostridium perfringens enterotoxin has been implicated as a cause of antibiotic associated diarrhoea[2] and diarrhoea by person to person transmission in hospitalised patients,[3] and also in elderly patients, not related to food borne outbreaks.[4 5] Another possible route of transmission is orally ingested spores from the environment or staff members in hospitals. In our study, only four (1.6%) patients were *C perfringens* toxin positive and only one of these had clinical diarrhoea. As Modi and Wilcox[1] recognise, there are considerable resource implications associated with routine screening for *C perfringens* enterotoxin. The apparent low incidence of *C perfringens* enterotoxin in patients with loose stools and the relatively mild symptoms displayed by positive patients suggests that routine screening may not be justified in our hospital.

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CORRECTION

Detection of human papillomavirus in large cell neuroendocrine carcinoma of the uterine cervix: a study of 12 cases. Grayson W, Taylor LF, Allard U, *et al*. *J Clin Pathol* 2002;**55**:108–14.

The journal apologises for the ommision of H A Rhemtula from the list of authors on the first page of this paper. The list of authors should have read as follows: Grayson W, Rhemtula HA, Taylor LF, Allard U, Tiltman AJ.