Infectious diarrhoea
– a laboratory perspective

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Infectious diarrhoea is a serious problem worldwide as well as in New Zealand. It is both a personal as well as a public health issue. The number of notifications is ever increasing despite public health initiatives. We know that the number of cases with a specific laboratory diagnosis are the tip of the iceberg and the majority of cases are never tested for a specific diagnosis.

Recently the Ministry of Health has recognised the importance of these infections by including them in *An Integrated Approach to Infectious Disease Priorities for Action 2002–2006*. For any such initiative to be successful it will be important for primary care workers, laboratories and public health agencies to work together.

In the USA the Infectious Diseases Society of America has formulated a Practice Guideline for the Management of Infectious Diarrhoea. In this document the expanding array of pathogens is emphasised as well as the pressure for cost containment.

Definitions

Diarrhoea is an increase in the frequency of stools as well as a decrease in consistency. The stool takes the shape of the container in which it is placed.

Acute diarrhoea is defined as having less than 14 days duration.

Persistent diarrhoea is that lasting for more than 14 days.

Chronic diarrhoea occurs when symptoms have been present for more than 30 days.

Infectious diarrhoea is diarrhoea which is caused by an infectious agent.

Infectious agents which commonly cause diarrhoea

Bacteria which cause diarrhoea are Campylobacter species, Salmonella species, Yersinia species, Shigella species, vero toxin producing E. coli (VTEC or STEC), Vibrio species and Clostridium difficile. Some other bacteria such as Aeromonas species and Plesiomonas species are also sometimes implicated as causes of diarrhoea.

Viruses are also a common cause of infectious diarrhoea, notably rotavirus, enteric adenoviruses, Norwalk and other small round viruses.

Intestinal parasites also cause diarrhoea. Giardia species and Cryptosporidium species are the commonest parasites implicated in New Zealand. Parasites that may be encountered in overseas travellers also include Cyclospora and Entamoeba histolytica (E. histolytica).

Laboratory testing for infectious causes of diarrhoea

Who should be tested?

When asked this question the answer from GPs varies from those who see testing as a waste of resources when the likelihood of offering specific treatment on the basis of the result is highly unlikely, to those who feel it is important to know what the cause of infectious diarrhoea is, both from a personal health viewpoint and also a public health perspective. The IDSA guideline suggested cases where the diarrhoea had lasted more than a day, especially if there was fever, bloody stools, systemic symptoms including dehydration, antibiotic use; also institutional exposure such as day care or recent hospitalisation. From a public health perspective it is also important to test food handlers.

It is important to know what the cause of infectious diarrhoea is, both from a personal health viewpoint and also a public health perspective

What test should be requested?

In most cases this will be a faecal culture. Most laboratories will culture for Campylobacter species, Salmonella species, Shigella species and Yersinia species. Culture for E. coli O157 (VTEC) is not performed on every sample and is directed by detection of blood in the stools, age and rural location – the criteria for this vary. Vibrio species also require special selective media which is used when clinical information indicates the sample is from an overseas traveller or raw seafood such as oysters have been eaten. Therefore clinical information which is useful to help guide culture is whether the patient has been overseas and whether they have consumed seafood.

Giardia and Cryptosporidium antigen tests have now become widely used. These enzyme immunoassay (EIA) techniques are more sensitive on a single sample than microscopy on a faecal concentration or acid fast stain on three samples. These two protozoan parasites are the commonest causes of parasitic diarrhoea in New Zealand. They should therefore

be requested when they could be the cause of infection. They are commonest in children, travellers including trampers and those who live in the country. Cryptosporidium is commonly associated with contact with young animals such as lambs and calves in spring time; it also causes outbreaks associated with contaminated swimming pools.

When samples are submitted for ova and parasites it is important that three samples are submitted. This is because the tests are less sensitive, because apart from concentrating the sample there is no amplification stage. The microscopist also relies on the parasite being in a suitable condition to recognise features essential for identification. To complicate things further, parasites are often shed intermittently.

Parasites that will be recognised from faecal concentrations are:

- E. histolytica cysts
- Giardia cysts
- Ova of helminths such as hookworm
- Blastocystis hominis.

Not all parasites causing diarrhoea will be detected using faecal concentration and microscopy. Trophozoites of E. histolytica, Giardia species, Dientamoeba fragilis and Cyclospora can only be reliably detected using staining techniques. For these it is often necessary to submit the three stool samples in fixative to preserve the delicate trophozoite stages of these organisms that facilitate their identification by microscopy.

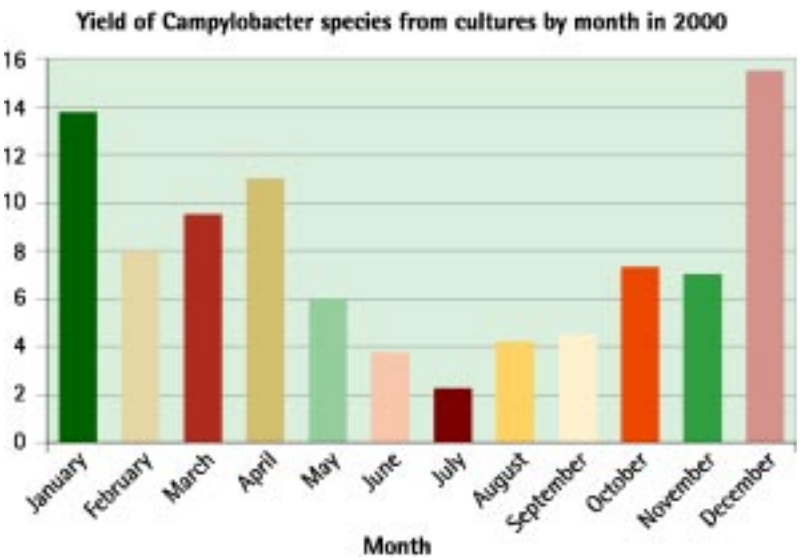
How many samples should be sent?

All stool samples except for those in fixative should be sent to the laboratory as soon as possible. When acute bacterial diarrhoea is suspected, one sample will detect the majority of cases. Therefore in most circumstances a single sample should be enough, however if symptoms continue, a second sample would be justified.

When giardiasis or cryptosporidiosis is suspected, a single sample tested by EIA has a sensitivity of 95–98%.

For ova and parasite examination three samples are essential. It is important that the samples are collected on three separate days, preferably with a free day in between, i.e. alternate days. If special stains are required it is important to request these and send some of the sample in fixative. *Note: Fixed samples are unsuitable for culture.*

Two or sometimes three samples are requested to clear patients who have had notifiable infections such as salmonella infection. It is important to culture more than a single sample because when the patient is no longer symptomatic the number of organisms in the sample will be less and therefore more difficult to detect.



Is it cost effective to send stool samples to the laboratory?

The IDSA guideline justifies the cost of testing stools for enteric pathogens on the basis that the information will be used for both personal health and public health. Calculations for cost effectiveness in the USA do not equate to the situation in New Zealand, however it is interesting to note the yields of tests from laboratories in the USA. The yield of bacterial enteric pathogens from 10 hospital laboratories was 5.6%; this was the highest yield reported. The yield of the specific pathogens was Salmonella species 1.8%, Shigella species 1.1%, Campylobacter species 2.3% and VTEC 0.4%. At Medlab South in Christchurch, which is a community laboratory, the yield of bacterial enteric pathogens in the year 2000 was a total of 10.9%. The yield of specific pathogens was Salmonella species 2.1%, Campylobacter species 7.9%, Yersinia species 0.4% and Aeromonas 0.5%. Aeromonas species have not been conclusively proven causes of diarrhoea. The difference in yield probably represents the difference between hospital and community laboratory patients, i.e. patients with more severe disease are seen in hospital. The low rate of isolation of Shigella species in New Zealand is interesting, however the reason for this is unclear.

The yield of protozoan parasites is also interesting. In the IDSA guideline the yield of Cryptosporidium species was 1.7% and Cyclospora 0.4%. In the year 2000 at Medlab South the yield of Cryptosporidium species was 0.7% and 0.8% for Giardia species. In New Zealand, community laboratories test for Giardia and Cryptosporidium species on request. Therefore the yield of these pathogens will be affected by the ordering patterns of referring doctors.

Recently a study on the prevalence of Clostridium difficile (C. difficile) as a cause of diarrhoea in-

dicated that this organism, which has mostly been associated with antibiotic associated diarrhoea and pseudomembranous colitis in hospitals, also causes diarrhoea in the community. Another finding has been that both C. difficile toxins A and B can cause disease and that laboratory testing should be performed which is capable of detecting both toxins otherwise some cases will be missed. A recent study performed at Medlab South has shown that both of these are important. A kit for the detection of both toxin A and B was evaluated against a toxin A detection kit. Ninety-one fluid stools containing leucocytes and erythrocytes on microscopy (but where no other infectious agent was identified) which had been stored at –70C, were tested with both kits. Thirty-six (40%) were positive for A+B whereas 12 were positive for toxin A. A second study using fresh samples of 142 loose stools with no other pathogen identified found 74 positive for C. difficile toxins A+B whereas 24 were positive for C. difficile toxin A alone.

When microscopic examination for erythrocytes and leucocytes was included, of the 70 samples with large numbers of erythrocytes and leucocytes, 51 samples were positive for C. difficile toxins A+B, whereas of those samples with no erythrocytes or leucocytes on microscopy, 23 were positive for C. difficile toxins A+B. This study indicates that it is important to test for both C. difficile toxins, but also that diarrhoeal disease caused by C. difficile is more common than previously thought.

The top three bacterial pathogens

Campylobacter species

Campylobacter jejuni is the bacterial pathogen which is most commonly isolated in cases of diarrhoea. The number of isolates is seasonal. Yields peak at 16% in the summer months and become much less in the winter. Epidemiological studies in New Zealand have shown that eating fresh chicken is associated with this infection. The infection may also occur with contact with young animals such as kittens and puppies. As in other countries, living in a rural area also carries a greater risk of infection. The severity of disease associated with campylobacter infection is variable. It ranges from asymptomatic to watery diarrhoea and or dysentery with blood and pus in the stools. It may cause pseudoappendicitis and mesenteric adenitis. Other sequelae such as reactive arthritis and Guillain Barre syndrome also occur. Although these latter complications are uncommon, because the number of cases in New Zealand is large, cases are seen on a regular basis.

Salmonella species

The second commonest bacterial pathogen is Salmonella. There are many serotypes of this organism. Recently there have been increased case numbers nationwide associated with the 'sparrow salmonella'. This was first associated with a significant number of cases in the South Island in 2001, but has now spread nationwide. Again, these infections are often zoonotic, at least initially, although Salmonella species also have potential for human-to-human spread. Acute diarrhoea is the most common symptom, but reactive symptoms, such as arthritis, also occur.

Yersinia enterocolitica

This organism causes acute diarrhoea often associated with abdominal pain. It is also cause of mesenteric adenitis and pseudoappendicitis. Reactive complications also occur and include reactive arthritis and Reiter's syndrome and erythema nodosum. This organism thrives at 4C and has occasionally caused transfusion related sepsis. Patients who have recently had this infection are therefore excluded from blood donation.

The recent Ministry of Health initiative *An Integrated Approach to Infectious Disease* has, in the section related to *Health Services* (DHBs, health providers, public health services) a comment: 'ensure effective reporting by providers/primary care and laboratories.' Certainly notification of these pathogens is a vital component of the public health follow-up of cases and the detection of outbreaks. Also, more research needs to be done before this stage to determine which patients should have samples tested, then which tests should be ordered in each instance to give the best outcome for the individual and the public health.