Original Article

Laboratory surveillance of communicable diseases: enteric pathogens

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Abstract

Laboratories represent a crucial link in the surveillance chain. Since only a small proportion of cases of enteric infections are asked to submit a stool sample, one needs to assess the practices for testing for enteric pathogens and their notification practices.

Five local laboratories participated in this study. This included a description of the laboratory practices; capacity for stool sample analysis; awareness of the notification system and the factors which could improve the system at laboratory level.

Three of the surveyed laboratories received a total of 2198 specimens during 2005, most of which were submitted without transport media. *Salmonella* and *Campylobacter* were usually sought in stool sample analysis. Tests for *E. coli* 0157 and Shiga toxin producing *E. coli* (STEC) were conducted in two of the laboratories. One of the laboratories conducted the test for *Cryptosporidium*, *Cyclospora* and *Microsporidia*. The test for rotavirus was conducted in two of the laboratories whilst one laboratory sent samples for testing for norovirus abroad when clinically or epidemiologically indicated.

All of the laboratories notified communicable diseases by regular mail. Feedback from the surveillance unit was welcomed by these laboratories, preferably through the website. Variations in testing for *E. coli*/STEC and parasites in different laboratories were observed.

Standardisation of testing procedures between laboratories is essential. The main limitation in laboratory reporting in the current system is the timeliness of notification which can be reduced by electronic data transfer from the laboratories to the national surveillance system. However, laboratory reporting does not replace the clinician's responsibility to notify cases.

Key words

Surveillance, laboratory reporting, enteric pathogens, electronic reporting

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Introduction

The limitations of passive surveillance systems compromise the accuracy and the quality of data.[1-4] Laboratories performing tests to isolate enteric pathogens from stool specimens are a crucial link in the surveillance chain.[5,6] It is known that a considerable number of stool specimens are negative for an aetiologic agent[7] and they are classified as unspecified in local statistics.[8] There are various reasons why a stool specimen from a case with symptoms of Infectious Intestinal Disease (IID) results in a negative finding. These include:

- Condition of specimen unsuitable for testing
- Double pathogen may lead to identification of only one pathogen
- Error in testing
- Long delay or inappropriate transport conditions leading to pathogens dying
- Number of pathogens below threshold level
- Symptoms of IID were due to a non-infectious cause
- Testing for pathogen responsible for illness not done
- Testing not done in laboratory
- Sensitivity of diagnostic method
- Characteristic of affected patient

Even when a pathogen is eventually identified, not all cases are reported to the surveillance chain. Hence they are excluded from national statistics, leading to under-estimates. The Disease Surveillance Unit (DSU) within the Public Health Department is responsible for the surveillance of infectious intestinal disease. This unit receives notifications from general practitioners, hospital physicians and laboratories. The majority of notifications received include cases which required hospitalisation or referral for stool culture analysis. To be included in the present surveillance system, an individual must first present to the health care provider who should notify the case.

Of those that present to the health care provider, only a small proportion of specimens are submitted for microbiological testing. Hence, the surveillance system captures only a tiny fraction of the infectious intestinal disease that is actually occurring in the community. In Malta, there is no published information on the practices that laboratories utilise to test for enteric pathogens; their analytical capabilities or their awareness to notification procedures. Therefore, a study was carried out to address these lacunae, and is described in this paper.

Methodology

Aims and objectives

The aim of the survey was to identify practices in laboratories that impact on the sensitivity of finding an aetiologic agent in submitted stool specimens.

It focused on:

- examination of some of the variables that influence whether a bacterial, viral or parasitic pathogen is identified in a stool specimen
- assessment of the laboratory capacity (availability, functionality, and level of sophistication)
- assessment on the awareness of the notification system
- the factors which may improve the notification system at this level

Definition and selection of participants

All laboratories (3 privately-owned and 2 managed by the government service) in the Maltese islands (Malta and Gozo) receiving stool specimens were invited to participate in the study. The list of licensed laboratories that have the permit to test human stool samples was obtained from the Department of Public Health which recommend the issuing of these licenses.[9]

Questionnaire design

The questionnaire design was based on the information required to meet the main aim and objectives of the study. The questionnaire included validated questions which were used in international studies in the United States[10] and Canada.[11] Questions included information on recording and transfer of data, type of laboratory, number of stool specimens received, types of pathogens tested for and variation in notification practices and awareness.

Pilot study

A pilot study using one laboratory was carried out to test the survey instrument, methodology and to identify any operational problems. Ambiguous questions were amended.

Field work

Laboratories were contacted by phone to introduce the study, confirm receipt of stool specimens and to identify contact persons. An appointment was made and the questionnaire was delivered by hand to the responsible person who in turn duly filled in the questionnaire.

Data entry and analysis

The data obtained from the questionnaires was fed into the Statistical Package for Social Sciences (SPSS) statistical analysis program, as soon as the forms were returned and the data was subsequently analysed.

Data protection and confidentiality

The University of Malta Research Ethics Committee approved the study. Consent from respondents to conduct the interview was obtained by telephone before the questionnaire was delivered. Data storage had security measures to limit access to the data management team only. Backups were stored securely. No identifiable data was released in any way.

Results

Participation rate

In accordance with the Medical and Kindred Act[12] all medical diagnostic laboratories require a license from the Superintendent of Public Health in order to operate in Malta. There were five licensed laboratories that perform analysis on stool specimens at the time of study. All of these laboratories participated in the study. Of these laboratories, one public laboratory was situated in hospital; the other public health laboratory covered public health samples; two laboratories were situated in private hospitals and another one in a private clinic.

Specimen transport for bacterial testing

A total of 2198 stool specimens were received during 2005 by three laboratories who responded to this part of the study, with the majority being in the main state hospital laboratory. They were routinely (>80% of times) received as faeces in a container without transport media; rarely (<20% of time) as faeces on ice or as a rectal swab with or without transport media. Only one of the laboratories received faeces in transport media. One laboratory sent stools for testing for bacteria to another laboratory as isolates on slopes. One laboratory occasionally sent samples for further phage typing abroad since this facility is not available in Malta. There were no changes in the off-site reference facilities in the previous two years for those who used the service.

Table 1: *Number of positive samples for bacterial pathogens from those analysed in three laboratories compared to total notified*

Pathogen	Number of samples analysed	Number of positive samples	% positive	Number of notified cases from laboratories
Salmonella	2064	89	4.3	99
Campylobacter	2140	75	3.5	96
E. coli	2967	24	0.8	23
Shigella	2064	0	0	0

Bacterial testing

Three of the five laboratories could provide the data on the percentage of positive bacterial samples since the database for the other laboratories was not set up to flag positive cases. The number of positive samples from these three laboratories was compared to the number which was actually notified to the DSU system[8] (Table 1).

Salmonella formed part of the routine screen for stool sample analysis.

Shigella was part of the routine screen at four of the laboratories with a total of 2,171 samples being tested in four laboratories in 2005.

Campylobacter was another routine screen. No direct non-culture methods are used by any of the laboratories for identification of *Campylobacter*.

Four of the laboratories test for *E. coli* 0157 and Shiga toxin producing *E. coli* (STEC). This was carried out routinely in two laboratories but only when a physician specifically requested such a test in another two laboratories. When testing for *E. coli* 0157 the laboratories used different media/methods including: MacConkey agar 9, chromagar 0157, immunomagnetic beads and Sorbitol-MacConkey agar. When sorbitol colonies are detected, different methods were used including a test to detect the 0157 antigen, a test to detect the H7 antigen, a biochemical test to identify the organism as *E. coli*, sending the isolate to a reference laboratory, performing verotoxin detection or a combination of some of these methods. Non-culture methods to screen for *E. coli* or STEC are not used by any of the laboratories.

None of the local laboratories processed faecal samples for *Vibrio* or *Yersinia*. One of these laboratories stated that they never had any requests and another would send samples abroad if requested. There was no Polymerase chain reaction (PCR)-based testing facility for bacterial pathogens in any of the laboratories.

Parasitic testing

During 2005, there were 1,444 faecal specimens submitted to four laboratories for enteric parasite screening. Samples were routinely (>80% of times) received as faeces in a container without transport media. All samples were analysed on site. Different procedures were used for testing for ova, cysts and parasites which included wet mounts before concentration or sedimentation, formalin-ethyl acetate concentration, modified ZN stain for *Cryptospridium parvum*, Weber modified trichrome stain for *Microsporidia* species, Lugol's iodine staining, buffered methylene blue and Lugol's iodine, Wheatley trichrome stain or a combination. Only one of the laboratories carried out testing for *Cryptosporidium*, *Cyclospora* and *Microsporidia*. Thirty eight out of 114 samples tested for *Cryptosporidium* in 2005 resulted in a positive test; none were positive for *Cyclospora* out of 114 tested and none were positive for *Microsporidia* out of 2 tested. In this laboratory, *Cryptosporidium* and *Cyclospora* testing was carried out on all liquid faecal specimens submitted for ova and parasites using an acid-fast stain, whilst *Microsporidia* testing was performed only when requested using chromotype stain.

Viral testing

Viral testing for enteric pathogens was only performed in two of the laboratories. Specimens were routinely received as faeces in a container without transport media. One of the laboratories sent samples for norovirus and rotavirus typing abroad. The only analysis made was for rotavirus which was performed using enzyme immunoassay. No other viral enteric pathogens were tested for.

Notification practices

All laboratories replied to this section of the questionnaire. A separate response was obtained from the virology section of the main state hospital leading to a total of six responses. All of these laboratories stated that they notified cases of communicable disease which they encountered in their analysis knowing that this is a legal obligation. Regular mail was the most common mode of sending notifications although one laboratory used fax and two used emails. The lag time from diagnosis to transmission of report varied from the same day up to one week or more.

Feedback was welcomed by all the laboratories, with the method most preferred by half of them being via a website; others preferred the annual report and the newsletter. Quarterly feedback was the most accepted frequency for feedback using internet or email as a medium for communication. Topics preferred were mainly information on outbreaks and epidemics as well as detection of imported diseases. Some welcomed information on trends in communicable diseases and vaccination activities.

All laboratories kept electronic records of the results of their investigations. However there was no flagging system whereby positive results could be electronically selected and there was no means for electronic linkage to the DSU database.

Discussion

Infectious intestinal disease is often self-limiting and is usually treated without a definitive diagnosis or confirmation of the aetiological agent. The focus group study on physicians[13] has identified this and we know that many of the cases occurring never come up for testing. However, from the public health point of view, information on the aetiological organism is very important for monitoring trends; as an early warning for the identification of outbreaks; for introducing control measures and for informing on policies. Whilst encouraging physicians to request stool samples, it is essential to assess the factors which could impact on the finding of the aetiological agent in local laboratories.

The response rate for the study was 100%. Being a small country with only a few laboratories it was essential to have all replies to the survey questionnaire so as to have an overall high response rate which would be representative. On the other hand it must be acknowledged that this may have been affected by the fact that the survey originated from a state health department. This may also have biased some of the results which laboratories might have been reluctant to admit, for example, to not notifying, despite the anonymity of the survey.

This study has shown that the main bulk of stools being submitted are sent to the microbiology laboratory of the main state hospital. The majority of persons who require hospital admission are admitted to this main state hospital which has a free of charge service for Maltese residents. Some prefer to go to a private hospital. The main state hospital laboratory also receives samples from the hospital's outpatients' department and from general practitioners both those working for the state and those in private practice.

The majority of samples were received without a transport medium. This is known to decrease pathogen viability especially if the sample is not tested immediately.[14] Hence, it would decrease the yield rate of pathogen isolation. Notification from the laboratory was usually carried out within the day of results issued but some reported more than a week later. This would also jeopardise the public health investigations which are required to be taken, particularly for outbreaks.

Although there was some consistency in testing for *Salmonella* and *Campylobacter*, there were variations in routing testing for other pathogens, especially for *E. coli* 0157/STEC. Testing for this pathogen was not routine as it is for *Salmonella* and *Campylobacter*, with the consequence that unless a physician suspects and requests this pathogen, it is not tested for. This would lead to an underestimation of the true rates.

No tests are performed for *Vibrio* or *Yersinia* in local laboratories. PCR testing for bacteria is not available either leading to a decrease the range of pathogen identification from stools. PCR has considerable advantages in terms of sensitivity, specificity, speed, range of targets and standardisation over conventional methodologies.

Similarly for parasites, different methods are used for analysis and many laboratories do not routinely test stool specimens for parasites. As a result, parasitic diseases are likely to be under diagnosed and under reported. This has also been reported from other studies carried out in other countries.[15,16]

The only enteric viral pathogen tested for in Maltese laboratories is rotavirus and only 2 laboratories conduct this. A study on IID in the community in Malta[13] has shown that a high proportion of cases in the community are caused by norovirus, and since this study has confirmed that this test is not performed routinely in Malta, it may explain why a large number of cases which are notified to the surveillance system remain unspecified (aetiological agent not identified). This great degree of viral under reporting has also been shown in UK figures, where only one in every 35 cases of rotavirus were reported[17], and in a Canadian laboratory study where only 14,051 samples were tested for viruses compared to 460,000 samples for bacteria.[16]

Another very important factor in the surveillance pyramid is that positive cases are notified. This study has revealed important aspects of notification by laboratories. All of the laboratories have confirmed that they do notify - but do they notify all cases? If the information of the percentage of positive cases was available from the laboratories, this could have been assessed.

Postal mail has been the traditional mode of sending notification forms. However, today with modern technology, different systems can be applied. Some are already using e-mail, although electronic security issues arise in this respect. For the main state hospital laboratory, the notifications are collected by hand on a daily basis, and in urgent cases the surveillance unit is informed by telephone. Automated systems for electronic data transfer would reduce the burden of data entry.[18-20] All laboratories in Malta have electronic records. An assessment of the cost-effectiveness of implementing such a system, including the costs of computer software and secure systems is required. Such an automated data transfer system would assist in generating hypothesis, monitoring trends, and detecting clusters or outbreaks.[21] A successful electronic reporting system needs public health coordination, standardised case definitions, careful planning and system support. With the introduction of electronic reporting in Hawaii, the total number of laboratory based reports doubled.[22] It has been known to enhance the quality of surveillance systems by simplifying reporting, improving completeness and increasing timeliness.[23-25]

Feedback has been seen as one of the most effective ways of improving notification.[13,26] The website was the most recommended route for this among laboratories as found in GPs, now that internet is widely available. The frequency of

feedback is realistic with quarterly feedback on variable subjects more related to the detection of cases and outbreaks rather than to prevention measures.

This study is subject to several limitations. Although the response rate was 100%, the number of laboratories involved is small. Yet, the study was able to reveal that the frequency of some pathogens may be underestimated because the laboratories do not do routine testing for them and physicians often do not request specific tests which are required. This contributes to another key area in the national surveillance system where cases are lost. Interventions to improve notification at laboratory level would assist in decreasing the lost cases which have been laboratory confirmed. Notification by laboratories does not replace the clinician's responsibility to notify cases especially where foodborne illness is suspected.

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