Future Perspectives on Infections Associated with Gastrointestinal Tract Diseases

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CHANGING BURDEN OF GASTROINTESTINAL DISEASE

In 2004, in the United States, there were 72 million presentations with a primary diagnosis of a digestive disease and 104 million presentations with combined gastrointestinal (GI) tract diseases and other diseases (Table 1). It was also found that those who are older tend to have more GI problems, there was no difference in the rates of digestive disease between the African Americans and the whites, and women were 20% more likely to present than men with digestive diseases. Thus, more than one-third (35%) of all presentations are for digestive diseases. In 2009, in the United States, the cancer statistics revealed 275,720 new cases of GI cancer, with colorectal and pancreatic cancer in the top 10 for both men and women. There were 135,830 deaths due to GI cancer, with colorectal, pancreatic, hepatic, and esophageal cancers in the top 10 for both men and women, except for esophageal cancer, which was only listed for men (Fig. 1). Furthermore, 2 of these 3 cancers that have an increasing mortality were GI tract cancers for both genders, with esophageal and hepatic cancers among men and pancreatic and hepatic cancers among women. Worldwide the rates of digestive diseases are staggering.

From 1979 to 1989, in the United States, a decrease was observed in the ambulatory care visits and hospital discharges for digestive diseases. These rates remained constant between 1990 and 1999, until 2000 when the rates climbed dramatically and was still increasing in 2004 (Fig. 2). During this period, substantial increases in the
| Digestive Disease          | Deaths, Underlying Cause<sup>a</sup> | Years of Potential Life Lost to Age 75 Years<sup>b</sup> | Ambulatory Care Visits, All-Listed Diagnoses<sup>c</sup> | Hospital Discharges, All-Listed Diagnoses<sup>c</sup> |
|---------------------------|---------------------------------------|---------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| All Digestive Diseases    | 236,164                               | 2,007,500                                               | 104,790,000                                            | 13,533,000                                              |
| All Digestive Cancers     | 135,107                               | 945,200                                                 | 4,198,000                                              | 726,000                                                 |
| Colorectal Cancer         | 53,226                                | 333,000                                                 | 2,589,000                                              | 255,000                                                 |
| Pancreatic Cancer         | 31,800                                | 206,800                                                 | 415,000                                                | 68,000                                                  |
| Esophageal Cancer         | 13,667                                | 113,800                                                 | 372,000                                                | 44,000                                                  |
| Gastric Cancer            | 11,253                                | 84,200                                                  | 141,000                                                | 31,000                                                  |
| Primary Liver Cancer      | 6323                                  | 72,400                                                  | 63,000                                                 | 33,000                                                  |
| Bile Duct Cancer          | 4954                                  | 32,900                                                  | —                                                      | 17,000                                                  |
| Gall Bladder Cancer       | 1939                                  | 10,900                                                  | —                                                      | 6,000                                                   |
| Cancer of the Small Intestine | 1115                               | 9300                                                   | —                                                      | 9000                                                   |
| Liver Disease             | 36,090                                | 559,100                                                 | 2,398,000                                              | 759,000                                                 |
| All Viral Hepatitis       | 5393                                  | 101,800                                                 | 3,510,000                                              | 475,000                                                 |
| Hepatitis C               | 4595                                  | 87,500                                                  | 2,747,000                                              | 419,000                                                 |
| Hepatitis B               | 645                                   | 11,800                                                  | 729,000                                                | 69,000                                                  |
| Hepatitis A               | 58                                    | 800                                                     | —                                                      | 10,000                                                  |
| GI Infections             | 4396                                  | 12,800                                                  | 2,365,000                                              | 450,000                                                 |
| Peptic Ulcer Disease      | 3692                                  | 19,700                                                  | 1,473,000                                              | 489,000                                                 |
| Pancreatitis              | 3480                                  | 42,800                                                  | 881,000                                                | 454,000                                                 |
| Disease                                      | Code | Rate | Incidence  | Mortality  |
|---------------------------------------------|------|------|------------|------------|
| Diverticular Disease                        | 3372 | 8600 | 3,269,000  | 815,000    |
| Abdominal Wall Hernia                       | 1172 | 6900 | 4,787,000  | 372,000    |
| Gastroesophageal Reflux Disease             | 1150 | 6000 | 18,342,000 | 3,189,000  |
| Gallstones                                  | 1092 | 4400 | 1,836,000  | 622,000    |
| All Inflammatory Bowel Disease              | 933  | 9100 | 1,892,000  | 221,000    |
| Crohn Disease                               | 622  | 7000 | 1,176,000  | 141,000    |
| Ulcerative Colitis                          | 311  | 2000 | 716,000    | 82,000     |
| Appendicitis                                | 453  | 5000 | 782,000    | 325,000    |
| All Functional Intestinal Disorders         | 423  | 2500 | 11,648,000 | 1,241,000  |
| Chronic Constipation                        | 137  | 900  | 6,306,000  | 700,000    |
| Irritable Bowel Syndrome                    | 20   | 0    | 3,054,000  | 212,000    |
| Hemorrhoids                                 | 14   | 200  | 3,275,000  | 306,000    |

*a Vital statistics of the United States.*  
*b The National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey.*  
*c The Healthcare Cost and Use Project Nationwide Inpatient Sample.*  

Data from Everhart JE, editor. The burden of digestive diseases in the United States. US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: US Government Printing Office, 2008; NIH Publication No. 09–6443; p. 6–7.
prevalence were observed for certain GI tract diseases, including gastroesophageal reflux disease (GERD) with an increase of 376 per 100,000 population, hepatitis C with 79 per 100,000 population, chronic constipation with 62 per 100,000 population, intestinal infections with 41 per 100,000 population, and pancreatitis with 23 per 100,000 population.1

The prevalence of digestive diseases around the world is enormous and varies from country to country (Table 2). Worldwide there has been a dynamic shift in the epidemiology of GI tract diseases, with some diseases such as peptic ulcer decreasing dramatically since the discovery of Helicobacter pylori infection and a larger number of conditions increasing, such as GERD, nonalcoholic fatty liver disease, diverticular disease, Barrett esophagus, cholelithiasis, alcoholic liver disease, hepatitis C, chronic pancreatitis, esophageal cancer and colorectal cancer.3–8 In conjunction with this increasing incidence of digestive diseases are the re-emergence of certain infectious agents (Box 1) (eg, cholera) and the identification of new agents (eg, H pylori, Laribacter, Campylobacter concisus), which are associated with GI tract diseases.9 Since the discovery of H pylori there has been an enormous interest in the relationship between microorganisms and GI tract diseases, including cancers.

CAUSE-AND-EFFECT ISSUES

One of the main issues associated with infections and disease is determining the relationship of the cause and effect. The landmark article by Sir Austin Bradford Hill in 1965 titled The environment and disease: association or causation? became widely known as the Bradford Hill’s criteria.10 There were 8 criteria that were required to
be met to determine a cause-and-effect relationship (Box 2). It is usually difficult to meet all these criteria, particularly when trying to find the cause-and-effect relationships between organisms in the small intestine or colon because of the large number of organisms living in these environments. Even for *H pylori* infection and the relationship with gastric cancer, although it is currently the only bacterium classified as a class I carcinogen, the evidence supporting this relationship is not complete in terms of Bradford Hill’s criteria.

**ORGANISMS ASSOCIATED WITH GI TRACT DISEASES**

There are a large number of organisms believed to be responsible for diseases of the digestive system. Some of these organisms are true pathogens, whereas others are merely commensal in nature and are unlikely to ever produce any pathologic condition. Table 3 shows the various types of microbes that are associated with diseases of the GI tract covered in this issue; it is by no means all-inclusive but provides the current magnitude of an ever-increasing field of research. At present, some of these diseases are only associated with a single group of organisms (eg, irritable bowel syndrome), whereas other diseases are affected by all groups of organisms (eg, appendicitis).

**FUTURE CHALLENGES**

There are a variety of methodological and technical issues related to infectious agents and their role in digestive diseases. For diseases of the colon, the major limitation remains the inability to completely identify these organisms. Identification of bacteria was mainly conducted using culture-based methods. Now, the focus in identification of bacteria is increasingly based on using molecular techniques. Many of these techniques allow the detection and identification of viable but nonculturable cells that are metabolically active but not reproducing. Gene sequencing using single-stranded RNA has been a key method in being able to elucidate multitudes of organisms that remain unknown. At present, there are approximately 9000 bacterial species, and this number is estimated as just the tip of the iceberg. The development of molecular
| Country/Region                        | Extrapolated Prevalence | Population Estimated Used |
|--------------------------------------|-------------------------|---------------------------|
| **Digestive Diseases in North America (Extrapolated Statistics)** |                         |                           |
| United States of America             | 64,776,924              | 293,655,405               |
| Canada                               | 7,170,854               | 32,507,874               |
| **Digestive Diseases in Europe (Extrapolated Statistics)**  |                         |                           |
| Austria                              | 1,803,256               | 8,174,762               |
| Belgium                              | 2,282,707               | 10,348,276               |
| Britain (United Kingdom)             | 13,295,008              | 60,270,708 for UK         |
| Czech Republic                       | 274,892                 | 10,246,178               |
| Denmark                              | 1,194,130               | 5,413,392               |
| Finland                              | 1,150,259               | 5,214,512               |
| France                               | 13,328,869              | 60,424,213               |
| Greece                               | 2,348,719               | 10,647,529               |
| Germany                              | 18,181,898              | 82,424,609               |
| Iceland                              | 64,845                  | 293,966                  |
| Hungary                              | 2,213,023               | 10,032,375               |
| Liechtenstein                        | 7375                    | 33,436                   |
| Ireland                              | 875,637                 | 3,969,558                |
| Italy                                | 12,806,795              | 58,057,477               |
| Luxembourg                           | 102,063                 | 462,690                  |
| Monaco                               | 7118                    | 32,270                   |
| Netherlands (Holland)                | 3,599,602               | 16,318,199               |
| Poland                               | 8,520,517               | 38,626,349               |
| Portugal                             | 2,321,502               | 10,524,145               |
| Spain                                | 8,885,465               | 40,280,780               |
| Country                        | Population | Expenditure |
|-------------------------------|------------|-------------|
| Sweden                        | 1,982,294  | 8,986,400   |
| Switzerland                   | 1,643,573  | 7,450,867   |
| United Kingdom                | 13,295,008 | 60,270,708  |
| Wales                         | 643,676    | 2,918,000   |

### Digestive Diseases in the Balkans (Extrapolated Statistics)

| Country                      | Population | Expenditure |
|------------------------------|------------|-------------|
| Albania                      | 781,942    | 3,544,808   |
| Bosnia and Herzegovina       | 89,913     | 407,608     |
| Croatia                      | 991,956    | 4,496,869   |
| Macedonia                    | 450,018    | 2,040,085   |
| Serbia and Montenegro        | 2,388,066  | 10,825,900  |

### Digestive Diseases in Asia (Extrapolated Statistics)

| Country                      | Population | Expenditure |
|------------------------------|------------|-------------|
| Bangladesh                   | 31,178,044 | 141,340,476 |
| Bhutan                       | 482,110    | 2,185,569   |
| China                        | 286,510,493| 1,298,847,624|
| East Timor                   | 224,834    | 1,019,252   |
| Hong Kong SAR                | 1,512,159  | 6,855,125   |
| India                        | 234,942,036| 1,065,070,607|
| Indonesia                    | 52,599,913 | 238,452,952 |
| Japan                        | 28,088,161 | 127,333,002 |
| Laos                         | 1,338,555  | 6,068,117   |
| Macau SAR                    | 98,224     | 445,286     |
| Malaysia                     | 5,188,782  | 23,522,482  |
| Mongolia                     | 606,907    | 2,751,314   |
| Philippines                  | 19,023,902 | 86,241,697  |
| Papua New Guinea             | 1,195,649  | 5,420,280   |
| Vietnam                      | 18,234,440 | 82,662,800  |
| Singapore                    | 960,417    | 4,353,893   |

(continued on next page)
| Country/Region                      | Extrapolated Prevalence | Population Estimated Used |
|-----------------------------------|-------------------------|---------------------------|
| Pakistan                          | 35,116,837              | 159,196,336              |
| North Korea                       | 5,006,812               | 22,697,553               |
| South Korea                       | 10,639,799              | 48,233,760               |
| Sri Lanka                         | 4,390,845               | 19,905,165               |
| Taiwan                            | 5,018,346               | 22,749,838               |
| Thailand                          | 14,308,570              | 64,865,523               |
| **Digestive Diseases in Eastern Europe (Extrapolated Statistics)** |                         |                           |
| Azerbaijan                        | 1,735,673               | 7,868,385                |
| Belarus                           | 2,274,379               | 10,310,520               |
| Bulgaria                          | 1,658,376               | 7,517,973                |
| Estonia                           | 295,955                 | 1,341,664                |
| Georgia                           | 1,035,417               | 4,693,892                |
| Kazakhstan                        | 3,340,522               | 15,143,704               |
| Latvia                            | 508,743                 | 2,306,306                |
| Lithuania                         | 795,860                 | 3,607,899                |
| Romania                           | 4,931,371               | 22,355,551               |
| Russia                            | 31,758,982              | 143,974,059              |
| Slovakia                          | 1,196,375               | 5,423,567                |
| Slovenia                          | 443,707                 | 2,011,473                |
| Tajikistan                        | 1,546,666               | 7,011,556                |
| Ukraine                           | 10,529,134              | 47,732,079               |
| Uzbekistan                        | 5,825,826               | 26,410,416               |
| **Digestive Diseases in Australasia and Southern Pacific (Extrapolated Statistics)** |                         |                           |
| Australia                         | 4,392,605               | 19,913,144               |
| New Zealand                       | 880,989                 | 3,993,817                |
### Digestive Diseases in the Middle East (Extrapolated Statistics)

| Country            | Population | Affected Population |
|--------------------|------------|---------------------|
| Afghanistan        | 6,289,781  | 28,513,677          |
| Egypt              | 16,790,606 | 76,117,421          |
| Gaza Strip         | 292,277    | 1,324,991           |
| Iran               | 14,890,412 | 67,503,205          |
| Iraq               | 5,597,358  | 25,374,691          |
| Israel             | 1,367,428  | 6,199,008           |
| Jordan             | 1,237,765  | 5,611,202           |
| Kuwait             | 497,988    | 2,257,549           |
| Lebanon            | 833,209    | 3,777,218           |
| Libya              | 1,242,261  | 5,631,585           |
| Saudi Arabia       | 5,690,280  | 25,795,938          |
| Syria              | 3,974,310  | 18,016,874          |
| Turkey             | 15,197,187 | 68,893,918          |
| United Arab Emirates | 556,745  | 2,523,915          |
| West Bank          | 509,824    | 2,311,204           |
| Yemen              | 4,417,249  | 20,024,867          |

### Digestive Diseases in South America (Extrapolated Statistics)

| Country | Population | Affected Population |
|---------|------------|---------------------|
| Belize  | 60,208     | 272,945             |
| Brazil  | 40,610,537 | 184,101,109         |
| Chile   | 3,490,578  | 15,823,957          |
| Colombia| 9,333,258  | 42,310,775          |
| Guatemala| 3,150,131 | 14,280,596          |
| Mexico  | 23,152,850 | 104,959,594         |
| Nicaragua| 1,182,299 | 5,359,759           |
| Paraguay| 1,365,742  | 6,191,368           |
| Peru    | 6,075,949  | 27,544,305          |
| Puerto Rico | 859,844 | 3,897,960           |
| Venezuela| 5,518,541 | 25,017,387          |

(continued on next page)
| Country/Region                  | Extrapolated Prevalence | Population Estimated Used |
|--------------------------------|-------------------------|---------------------------|
| Angola                         | 2,421,739               | 10,978,552<sup>b</sup>    |
| Botswana                       | 361,595                 | 1,639,231<sup>b</sup>     |
| Central African Republic       | 825,547                 | 3,742,482<sup>b</sup>     |
| Chad                           | 2,104,090               | 9,538,544<sup>b</sup>     |
| Congo Brazzaville              | 661,332                 | 2,998,040<sup>b</sup>     |
| Congo Kinshasa                 | 12,864,050              | 58,317,030<sup>b</sup>    |
| Ethiopia                       | 15,736,007              | 71,336,571<sup>b</sup>    |
| Ghana                          | 4,578,756               | 20,757,032<sup>b</sup>    |
| Kenya                          | 7,275,464               | 32,982,109<sup>b</sup>    |
| Liberia                        | 747,934                 | 3,390,635<sup>b</sup>     |
| Niger                          | 2,506,000               | 11,360,538<sup>b</sup>    |
| Nigeria                        | 3,915,519               | 125,750,356<sup>b</sup>   |
| Rwanda                         | 1,817,354               | 8,238,673<sup>b</sup>     |
| Senegal                        | 2,393,855               | 10,852,147<sup>b</sup>    |
| Sierra Leone                   | 1,297,916               | 5,883,889<sup>b</sup>     |
| Somalia                        | 1,831,897               | 8,304,601<sup>b</sup>     |
| Sudan                          | 8,635,623               | 39,148,162<sup>b</sup>    |
| South Africa                   | 9,804,809               | 44,448,470<sup>b</sup>    |
| Swaziland                      | 257,920                 | 1,169,241<sup>b</sup>     |
| Tanzania                       | 7,956,793               | 36,070,799<sup>b</sup>    |
| Uganda                         | 5,821,380               | 26,390,258<sup>b</sup>    |
| Zambia                         | 2,432,137               | 11,025,690<sup>b</sup>    |
| Zimbabwe                       | 809,969                 | 12,671,860<sup>b</sup>    |

Abbreviation: SAR, special administrative region.

<sup>a</sup> US Census Bureau, population estimates, 2004.

<sup>b</sup> US Census Bureau, international database, 2004.
Box 1
List of the National Institute of Allergy and Infectious Diseases on emerging and re-emerging diseases

Group I: pathogens newly recognized in the past 2 decades
Acanthamebiasis
Australian bat Lyssavirus
Babesia, atypical
Bartonella henselae
Ehrlichiosis
Encephalitozoon cuniculi
Encephalitozoon hellem
Enterocytozoon bieneusi
H pylori
Hendra or equine morbillivirus
Hepatitis C
Hepatitis E
Human herpesvirus 8
Human herpesvirus 6
Lyme borreliosis
Parvovirus B19

Group II: re-emerging pathogens
Enterovirus 71
Clostridium difficile
Mumps virus
Streptococcus, group A
Staphylococcus aureus

Group III: Agents with bioterrorism potential
National Institute of Allergy and Infectious Diseases (NIAID): category A
Bacillus anthracis (anthrax)
Clostridium botulinum toxin (botulism)
Yersinia pestis (plague)
Variola major (smallpox) and other related poxviruses
Francisella tularensis (tularemia)
Viral hemorrhagic fevers
Arenaviruses: lymphocytic choriomeningitis virus, Junin virus, Machupo virus, Guanarito virus, Lassa fever
Bunyaviruses: Hantaviruses, Rift Valley fever, Flaviviruses, dengue virus
Filoviruses: Ebola, Marburg
NIAID: category B
Burkholderia pseudomallei
Coxiella burnetii (Q fever)
Brucella species (brucellosis)
Burkholderia mallei (glanders)
Chlamydia psittaci (psittacosis)
Ricin toxin (from Ricinus communis)
Epsilon toxin of Clostridium perfringens
Staphylococcus enterotoxin B
Typhus fever (Rickettsia prowazekii)
Food- and waterborne pathogens
Diartheagenic Escherichia coli
Pathogenic vibrios
Shigella species
Salmonella
Listeria monocytogenes
Campylobacter jejuni
Yersinia enterocolitica
Viruses (Caliciviruses, Hepatitis A)
Protozoa: Cryptosporidium parvum, Cyclospora cayetanensis, Giardia lamblia, Entamoeba histolytica, Toxoplasma
Fungi
Microsporidia
Additional viral encephalitides: West Nile virus, La Crosse virus, California encephalitis virus, Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, Western equine encephalitis, Japanese encephalitis virus, Kyasanur forest virus
NIAID: category C
Emerging infectious disease threats such as Nipah virus and additional hantaviruses
NIAID priority areas
Tick-borne hemorrhagic fever viruses: Crimean-Congo hemorrhagic fever virus
Tick-borne encephalitis viruses
Yellow fever
Multidrug-resistant tuberculosis
Influenza
Other rickettsias
Rabies
Prions
Chikungunya virus
Severe acute respiratory syndrome–associated coronavirus
Antimicrobial resistance, excluding research on sexually transmitted organisms
  Research on mechanisms of antimicrobial resistance
  Studies of the emergence and/or spread of antimicrobial resistance genes within pathogen populations
methods offers great promise not only in research and development but also in the diagnostic setting (eg, stool samples) (Table 4).\textsuperscript{11,12} Clearly, metagenomics, in which genetic material is directly retrieved from environmental sources, will play a critical role in the future development of determining infectious agents of the GI tract. The use of high-throughput technology has already produced important findings in relation to the GI tract microflora, including the differences between adults and children, with numerous uncultured organisms being the crux of the normal human adult gut flora which remain stable but other organisms change depending on environmental and genetic factors, whereas in infants there appear to be a constant transformation of organisms over time (Figs. 3 and 4).\textsuperscript{11} There have been several new detection methods developed, with some of these using nanoscale electrochemical detectors and others using DNA sensors (extrachromosomal DNA).\textsuperscript{13} The use of stable-isotope probing is also being investigated, but even this technique has limitations.\textsuperscript{14} Although these technologies are increasing the understanding of the gut microflora, there remains large gaps of knowledge regarding the metabolic functions of these organisms and the relationship they have with human GI disease. These will be extremely fruitful areas of research and development in the coming years.

| Box 2 |
|---|
| **Bradford Hill's criteria for causality** |
| Consistency: The association is consistent when results are replicated in studies in different settings using different methods. |
| Strength: This is defined by the size of the risk as measured by appropriate statistical tests. |
| Specificity: This is established when a single putative cause produces a specific effect. |
| Dose-response relationship: An increasing level of exposure (in amount and/or time) increases the risk. |
| Temporal relationship: Exposure always precedes the outcome. |
| Biologic plausibility: The association agrees with currently accepted understanding of pathobiologic processes. This criterion should be applied with caution. |
| Coherence: The association should be compatible with existing theory and knowledge. |
| Experiment: The condition can be altered by an appropriate experimental regimen. Experiment is possibly the most important support for a causal relationship. |
| GI Tract Disease   | Bacteria                                      | Virus                                      | Parasite                   | Fungi                           |
|-------------------|----------------------------------------------|--------------------------------------------|----------------------------|---------------------------------|
| Esophageal Cancer | \( \alpha \)-hemolytic streptococcus, \( \beta \)-hemolytic streptococcus, Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides sp, Clostridium sp, coagulase-negative Staphylococcus, Corynebacterium sp, Escherichia coli, Fusobacteria sp, Haemophilus influenzae, Lactobacillus sp, Neisseria catarrhalis, nonhemolytic streptococcus, Peptococcus, Pneumococcus, Proteus mirabilis, Staphylococcus albus, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans, Candida albicans Mycobacterium avium, Mycobacterium tuberculosis | Cytomegalovirus, Epstein-Barr virus, Herpes simplex virus, Varicella-zoster virus | Cryptosporidium | Histoplasma capsulatum, |
| Gastric Cancer    | \( H \) pylori                              | Epstein-Barr virus                         | —                          | —                              |
| Cholangiocarcinoma| —                                            | Hepatitis C virus, Hepatitis B virus       | Clonorchis sinensis, Opistochus vivarini | —                              |
| Gall Bladder Disease | \( E \) coli, \( H \) pylori, Helicobacter sp, Enterobacteriaceae, Leptospira, Salmonella enteritidis, Salmonella typhi, Staphylococcus aureus, Micrococcus sp | Cytomegalovirus, Epstein-Barr virus, Dengue virus | C sinensis, O vivarini, Ascaris lumbricoides, Dolosigranulum pigrum | Actinomyces sp, Candida sp, |
| Hepatocellular Carcinoma | —                                          | Hepatitis B virus, Hepatitis C virus       | —                          | —                              |
| Diarrheal Disease | Associated Organisms | Other Pathogens | Infections Associated with GI Tract Diseases |
|-------------------|----------------------|-----------------|--------------------------------------------|
| Acute Pancreatitis | Mycoplasma pneumoniae, *S typhi*, Leptospira, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, Campylobacter jejuni, *M tuberculosis*, *M avium*, Legionella sp, Brucellosis, Actinomyces, Nocardia | Measles virus, Coxsackie B virus, hepatitis B virus, Cytomegalovirus, herpes simplex virus, varicella virus, human immunodeficiency virus, Epstein-Barr virus, vaccinia, rubella, adenovirus | Aspergillus sp, Cryptococcus neoformans, Coccidioides immitis, Paracoccidioides brasiiliensis, Histoplasma capsulatum, Pneumocystis carinii |
| Small Intestinal Bacterial Overgrowth | Streptococcus sp, *E coli*, Staphylococcus sp, Micrococcus sp, Klebsiella sp, Methanobrevibacter smithii, Bacteroides sp, Firmicutes sp | — | — |
| Irritable Bowel Syndrome | Salmonella sp, Campylobacter sp, Shigella sp, Enterobacteriaceae, Clostridia | — | — |
| Inflammatory Bowel Disease | *E coli*, *M avium*, Streptococcus sp, Clostridia, Actinobacteria, Proteobacteria, Clostridium leptum, Faecalibacterium prausnitzii, Bacteroides, Fusobacteria | — | — |
| Appendicitis | *Y enterocolitica*, *Y pseudotuberculosis*, Actinomyces israelii, Mycobacterium, C jejuni, Clostridium difficile, Salmonella sp, B fragilis | Adenovirus, cytomegalovirus, measles virus (rubeola virus) | A lumbricoides, Enterobius vermicularis, Strongyloides stercoralis, Schistosomiasis haematobium, Entamoeba histolytica, Trichuris sp |
| Colorectal Cancer | Helicobacter hepaticus, Enterococcus faecalis, Streptococcus bovis, H pylori, Clostridium septicum, *E coli*, Streptococcus sanguis, Streptococcus salivarius | Human papillomavirus, JC virus, Epstein-Barr virus, cytomegalovirus | — |
| Instrument | Method | Number of Samples | Time Required |
|------------|--------|-------------------|---------------|
| ABI PRISM 6100 nucleic acid PrepStation (Applied Biosystems) | Silica membrane bind/elute protocols with vacuum processing (RNA and DNA) | Up to 96 | 30 min |
| ABI PRISM 6700 automated nucleic acid workstation (Applied Biosystems) | Silica membrane bind/elute protocols with vacuum processing (RNA and DNA) | Up to 96 | 90 min |
| BioRobot EZ1 workstation (QIAGEN) | Silica membrane bind/elute protocols using magnetic-particle handling (RNA and DNA) | 1–6 | 15–20 min |
| iPrep Purification Instrument (Invitrogen) | Based on a unique, ionizable nucleic acid-binding ligand whose charge can be switched based on the pH of the surrounding medium (DNA) | Up to 12 | 18 min |
| KingFisher ML/96 (Thermo Scientific) | Silica membrane bind/elute protocols using magnetic-particle handling (RNA and DNA) | 1–96 | 20–30 min |
| MagNA pure compact/LC (Roche Applied Science) | Silica membrane bind/elute protocols using magnetic-particle handling (RNA and DNA) | 1–32 | 15–40 min |
| Maxwell 16 Instrument (Promega) | Silica membrane bind/elute protocols using magnetic-particle handling (RNA and DNA) | Up to 16 | 30 min |
| NucliSens miniMAG (BioMérieux) | Silica membrane bind/elute protocols using magnetic-particle handling (RNA and DNA) | Up to 12 | 45 min |
| QIAcube (QIAGEN) | Silica membrane bind/elute protocols with built in centrifuge (RNA and DNA) | Up to 12 | 60 min, user-developed protocols |
| X-Tractor Gene RNA/DNA Extraction System (Corbett Life Science) | Silica membrane bind/elute protocols with vacuum processing (RNA and DNA) | 8–96 | 1 h |

*Data from Barken KB, Haagensen JA, Tolker-Nielsen T. Advances in nucleic acid-based diagnostics of bacterial infections. Clin Chim Acta 2007;384:1–11.*
Fig. 3. High-throughput analysis of human GI tract microbiota via brute force sequencing and phylogenetic microarray analysis. SSU rRNA, small subunit ribosomal RNA. (From Zoetendal EG, Rajilic-Stojanovic M, de Vos WM. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut 2008;57:1605–15; with permission.)

Fig. 4. Metagenomics and other community-based “omics” approaches. SSU rRNA, small subunit ribosomal RNA. (From Zoetendal EG, Rajilic-Stojanovic M, de Vos WM, High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut 2008;57:1605–15, with permission.)
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