Tracing pathogens in the food chain

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201 **Protective cultures, antimicrobial metabolites and bacteriphages for food and beverage biopreservation** *Christophe Lacroix*

Preface

Understanding the epidemiology of pathogens in production chains and processing environments is highly important for food safety control. Epidemiological surveillance is also an essential part of a food safety programme. The ever increasing possibilities of modern tools and techniques offer new options to trace unwanted organisms in the chain and take adequate timely measures. The original idea of combining experts from all fields of the food chain led to a combined editorship of the book that covers these aspects. The result is a comprehensive collection where epidemiology and surveillance set the scene for the application of the novel technologies for tracing pathogens in the food chain. We hope that this book will fill a critical void in the scientific literature and will prove of interest to food producers, governmental organizations, and the international scientific community. The editors would like to express here their gratitude to all who have contributed to the volume and to the excellent support at Woodhead Publishing. Acknowledgements also go to our families who had to endure our late-night readings or early weekend rises.

Stanley Brul, Pina M. Fratamico and Tom A. McMeekin

1

Introduction

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Abstract: The presence of microorganisms in our day-to-day lives is briefly put into perspective with a strong focus on those bacteria that cause food safety concerns. From considerations on their initial presence, we move to their identification with state-of-the-art molecular tools and close the loop with attention to surveillance and microbial behaviour in specific chains. The contemporary concept in (predictive) food microbiology of the need to transform mechanistic data as much as possible to models at different organisational levels of biological structure and function is introduced.

Key words: foodborne pathogens, tracing and tracking microorganisms in the food chain, modelling microbial behaviour, systems analysis of microbial food preservation.

1.1 Microbes and the food chain

The world of microorganisms holds many promises and threats for the food manufacturer. On the one hand, the cells provide a rich source of functional molecules that can be introduced to the benefit of consumers into the diet through (food) fermentation (Nout, 2009; Kleerebezem *et al*., 2010). On the other hand, the presence and proliferation of unwanted microorganisms can have consequences ranging from harmless but economically damaging food spoilage to dangerous food safety incidents (Havelaar *et al*., 2010; Rajkovic *et al*., in press). The aim of the food producer is to detect, identify, and enumerate the organisms of interest as soon as possible using the best methods that are currently available. Measures to deal with the unwanted presence of microbes can then rapidly be taken. For governmental organisations, this implies improved public health assurance (Havelaar *et al*., 2010) while for the producer it means that standard operating procedures in food manufacturing, as well as adequate immediate action, including recalls if necessary, can be applied in an optimised manner (Jacxsens *et al*., 2009). In all cases the consumer benefits from the improved response time and response quality. This book provides a topical overview

of various important aspects involved, ranging from an assessment of the full food chain to genomics-based analysis of the isolated relevant microorganisms. Below we first give a bird's eye view of the various parts of the book and then provide a view on integration of the current topics in a systems approach using quantification tools at the various levels of complexity in the chain. The approach closely matches the views expressed in the European Technology Platform Food for Life (http://cordis.europa.eu/technology-platforms/pdf/foodforlife.pdf).

1.2 Where and in what 'state' noxious microbes are in our food chain

Here we highlight some of the main considerations of the various parts of the book. We will not dwell extensively on each individual chapter but rather highlight points to arouse interest in the reader who can then find the detail in the various chapters themselves.

1.2.1 Foodborne pathogen surveillance and outbreak investigation

This part deals with practical issues regarding outbreak detection and surveillance (see also the review by Pires *et al*., 2009). Dr Fisher sets the scene by indicating the crucial role of an integrated approach to surveillance. He makes a plea for collaboration between services active on the human, veterinary and food aspects of outbreak surveillance and investigation. Dr Stein of the WHO provides an integrated view on outbreak detection and investigation. The data clearly shows the different challenges that the Western world faces compared to developing countries. Many of the issues can be addressed by available measures and appropriately applying well-known procedures. Also, sometimes mainly, economic considerations can be prime factors in determining the success of preventative, as well as curative strategies (Buzby and Roberts, 2009; Palou *et al*., 2009). The chapter by Hald and Pires discusses strategies for addressing the attribution of foodborne infections along the chain. They elegantly show the various analyses, from epidemiological considerations to intervention studies and application of expert knowledge as primary input. Finally, a parameter already mentioned but still often imprudently easily tucked away is economic cost. The chapter by Buzby elaborates on such economic costs versus the global burden of foodborne disease. Managerial choices and scenarios are spelled out in cost–benefit scenarios.

1.2.2 Subtyping of foodborne pathogens

The characteristics of foodborne pathogens may well vary over time just as they do for any microbial population. Hence tools to monitor such change that are relevant at the physiological level are of major importance (discussed in Davidsen *et al*., 2009; Hornstra *et al*., 2009). The chapter by Gebreyes and Thakur sets the scene, and the use of phenotypic markers to assess behaviour at the cellular level

is described. Cooper and coworkers then describe pulsed-field gel electrophoresis and related molecular methods for subtyping of bacteria. Pagotto adds a number of new 'pearls on the string' of subtyping tools. At all instances it is crucial that methods are validated and referenced. The chapter by Hyytia-Trees and Ribot does just that: referencing and standardization of (new) bacterial subtyping tools for use in the food chain.

1.2.3 Molecular methods, genomics and other emerging approaches in the surveillance and study of foodborne pathogens

As any other biology discipline, microbiology has recently made major leaps forward through the 'omics' revolution (Zhang *et al*., in press). Transformation of the new data into knowledge as well as application in various applied settings is currently rapidly developing (Borneman *et al*., 2007; Brul *et al*., 2008). For applications in any field and thus also in food microbiology proper nucleic acid extraction protocols are essential. The chapter by Wagner and colleagues reiterates this point. In many cases of tracing pathogens in the food chains is all about finding the needle in the haystack, or, in other words, identifying the presence and pathogenic characteristics of low absolute numbers of microorganisms. The chapter by van der Vossen and coworkers next shows how various molecular typing methods can find practical application in surveying for *Campylobacter* occurrence in foods. Genome-wide typing methods are increasingly used as *Campylobacter* remains a major food safety concern. Costs for full genome sequencing are rapidly declining so that this is an increasingly viable option (Petrosino *et al*., 2009).

The application of comparative genome-wide analysis is further discussed by Stabler *et al*. in a more generic sense. Non-nucleic acid methods that emerge for detection and characterisation are extensively discussed by Bowman from the University of Tasmania. Specific typing based on the presence of virulence genes is introduced by Wassenaar in Chapter 15. Toxigenic and spoilage bacterial spore-formers are a prime concern and economic problem to industry and society. Maybe even more than vegetative cells, spore-forming organisms often set the boundaries of what is possible in terms of food preservation treatments (van Zuijlen *et al*., in press). While minimisation of heat application from sterilisation to near-pasteurisation values is highly desirable from a consumer's food quality perspective, food safety has to remain guaranteed throughout. The chapter by Van Zuijlen gives an industrial look at how ribotyping methods can be deployed to address recurring issues with bacterial spore-formers in the food manufacturing industry.

Finally, integration of microbiology data, complex systems analyses and stochastic models is discussed in the framework of 'Bio-tracing', an EU sponsored approach.

1.2.4 Tracing pathogens in particular food chains

The last part of the book is devoted to finding microbial pathogens in particular chains. An account is given of events in meat and game production by Whyte

et al. This is a type of food chain that traditionally has significant issues with food safety. Fish production and the pathogen presence/behaviour in chicken is discussed by Lunestad *et al*. and Hiett *et al*., respectively. Dairy, shellfish and fresh produce are chains of generic interest that complete the overview given in the book. In all chains it is important to analyse the environmental conditions to and identify generically relevant parameters. These can be used to define better test conditions for more fundamental studies on the mechanisms involved in maintenance of microorganisms in a food chain environment. Such mechanistic basis will improve the robustness of predictive models of microbial behaviour in foods (McMeekin *et al*., 2007, 2010).

1.3 Towards integration

1.3.1 Modelling cellular behaviour

Many scientists in the field advocate an integrated 'systems' approach in tracing (and tracking) microorganisms in foods. Safety by design is seen as the way forward. Improved knowledge of the microorganisms under study is instrumental in providing novel options to test for their presence and behaviour. Systems biology is the analysis routine that is more and more applied in which the cycle between experiment and functional integrative genomics analysis of behaviour in a food-related environment is the key. In Fig. 1.1, this view is shown schematically for the analysis of the behaviour of bacterial spores. Clearly for this tool to be of use to food microbiology, models are needed that can operate at many different levels of complexity (Brul *et al*., 2008; McMeekin *et al*., 2007, 2010). Also, it is crucial that stochastic elements are introduced as it is more and more clear that heterogeneous behaviour, assessed on the basis of physiological end-points (growth, death, lag-time), of cells from genetically homogeneous populations is more the rule than the exception (Stringer *et al*., 2009).

Such heterogeneity is often ruled by fluctuations in gene expression (Pin *et al*., 2009). To assess the importance of various genes in regulatory pathways, sensitivity analysis regarding their control on signal output is key (Veening *et al*., 2008; Hornstra *et al*., 2009). To test for the expression levels of proteins, antibody-based staining is possible generally preceded by analysis of the behaviour of model organisms. The latter often allows for the full scope of genomics techniques to be used. In all this, data analysis is crucial. What will be needed increasingly are 'biological engineers' who are specialists with full appreciation of the biological complexity as well as a quantitative view on cellular biochemistry and molecular biology.

1.3.2 Modelling food microbiology in the framework of the food chain

When analysing the importance of molecular cell biology in food microbiology it is crucial to couple the stochastic data at the cellular level to the probability of cellular distribution over the chain (Havelaar *et al*., 2010). The data will have to

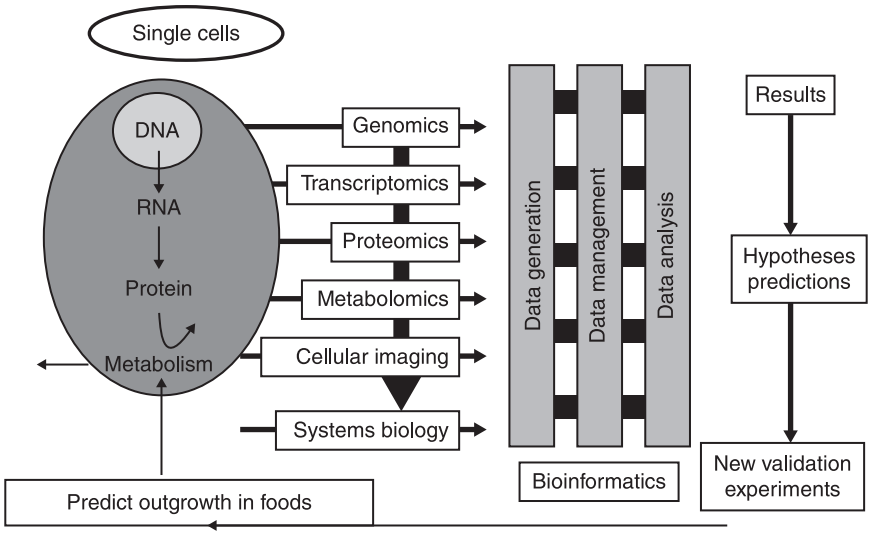

Fig. 1.1 A schematic representation of the sequence of events in systems biology as they pertain to food microbiology. Initially data is obtained at the various 'omics' levels. Such data needs to be complemented with proper image analysis or equivalents to assess the heterogeneity in expression of (various) signalling pathways in a genetically homogeneous population. The level of heterogeneity is increasingly seen as an important parameter determining the developmental state of (most) of the microorganisms in a genetically homogeneous population. Integration of the omics data as well as the single cell analysis data by 'biological engineers' (see main text) should provide models and lay the foundation for a systems biology approach to microbial food safety. Models are tested and improved through iterative cycling.

be input for techniques and approaches that are being developed for risk-benefit evaluation where consumer demands are well balanced against food safety risks. To profit from the improved biological understanding, it will be crucial to put the data on microbial cell physiology in relation to microbial exposure likelihood and disease risks at the consumer level. In doing so, the risk-benefit evaluation framework will have to be adapted to incorporate this new data efficiently. Here sustainable processing, preservation, packaging and logistic systems have to be developed. Balancing these often counteracting drivers of microbial food quality and safety requires developing models that can 'talk to each other'. That is meaning that improvements in the quantitative prediction of microbial physiology can be 'read' by models of cellular distribution and manufacturing, as well as by epidemiological risk assessment models (Havelaar *et al*., 2010). Going one step further, biological models to establish the mechanistic basis of survival and virulence in the host (consumer) will be welcome to enhance the robustness of epidemiological models. Such models should also aim at being (more) proactive in identifying emerging pathogens and their characteristics.

To consider all these boundary conditions systematically is highly complex to set priorities in food microbiology research and continuous challenge of the criteria used will be important to reach a common practice.

In conclusion, in the overall context of the food chain, the value of tracing (and tracking) pathogens will only become proactively beneficial to food production if progress in molecular microbial physiology is translated by 'biological engineers' to outputs transferable to food engineers who can use these models to improve robustness and predictive powers of their food chain models. In these models either optimal safety settings or optimal quality settings may be taken as input. Thus, the engineering approach to the various areas of life science will be the key towards integration and effectively generating a 'systems approach' to microbial stability. This type of approach is indeed much advocated by the European Technology Platform Food for Life (Fig. 1.2 and http://cordis.europa.eu/technology-platforms/pdf/foodforlife.pdf).

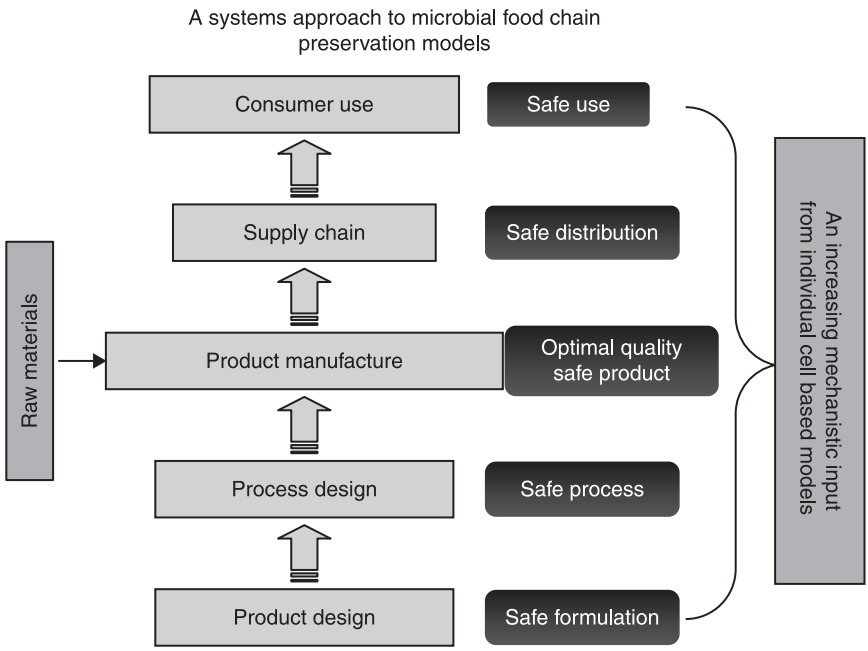

Fig. 1.2 The view on current and near future developments in modelling of microbial behaviour throughout the food chain. Clearly to develop models at the various levels of complexity of the chain will require input from agriculture to medicine and from consumer to producer (fork to farm). Such models will increasingly use individual cell based models as input. Modified from the European Technology Platform Food for Life (http://cordis.europa.eu/technology-platforms/pdf/foodforlife.pdf).

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2

Surveillance for foodborne pathogens in humans

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Abstract: Surveillance, in its short form, has been defined as 'information for action'. This chapter describes some of the surveillance methods that can be used and gives examples of their usage on a national and international basis. It describes some of the international outbreaks that have been identified with a range of pathogens and foodstuffs involved. It summarises the limitations of surveillance activities and some future trends. Most importantly, it emphasises the importance of collaboration between the human, veterinary and food aspects of foodborne surveillance to coordinate successful activities in this area.

Key words: human surveillance methods, international outbreaks, surveillance methods, International Health Regulations.

2.1 Introduction

Surveillance has been defined as the ongoing systematic collection, collation and analysis of data and the prompt dissemination of the resulting information to those who need to know so that an action can result. This can be more succinctly phrased as 'information for action' (Berkelman and Buehler, 1991). Information for action is most obvious in terms of foodborne disease surveillance in the identification of foodborne outbreaks and their causes and the implementation of public health measures to remove the vehicle of infection from the market place and hence stop further cases of illness. Although this is an immediate and effective method of stopping outbreaks, the longer term accumulation of evidence is often necessary for effective strategic intervention measures to be undertaken. An example of this is the knowledge that *Salmonella enterica* subsp. *enterica* serovar Enteritidis phage type (PT) 4 in the UK in the late 1980s and early 1990s was present in breeder and layer chicken flocks; however, it was not until almost ten years after

it was recognised that intervention measures were implemented. The intervention measure initially introduced was the vaccination of breeder flocks in the UK to prevent onward transmission of *S.* Enteritidis PT4. While this showed a small but short-lived effect, it was not until the vaccination of layer flocks was brought in that a significant reduction in the level of human infections was achieved (Fig. 2.1). This has had an impact on eggs produced for domestic consumption, but it has not prevented problems with eggs imported into the UK (Little *et al*., 2007). This demonstrates one of the difficulties with current food production and, more pertinently, consumption. The desire for seasonal foods all the year round has led to the increased shipping of products around the globe to fill this need. Hence, products can be harvested in countries where control measures can be less stringent and the potential for contamination much greater. Similarly, manufacturing practise has changed from products being made locally to being made at one site and shipped nationally, internationally or globally due to economies of scale; again, this allows the possibility of a contaminated product being distributed to consumers in many countries. These factors have led to many international outbreaks of foodborne pathogens occurring far from the source of the product. To demonstrate the diversity of the problem of international foodborne outbreaks, a selection of the pathogen/food combinations that have been identified are: *Salmonella*/ready to eat snacks (Killalea *et al*., 1996), *Salmonella*/Halva (de Jong *et al*., 2001), *Salmonella*/peanuts (Kirk *et al*., 2004), *Salmonella*/chocolate (Werber *et al*., 2005), *Salmonella*/fresh basil (Pezzoli *et al*., 2008), *Escherichia coli*/spinach (Grant *et al*., 2008; Wendel *et al*., 2009), *Salmonella*/cooked meats (O'Flanagan *et al*., 2008), norovirus/raspberries (Maunula *et al*., 2009) and *Shigella*/baby corn (Lewis *et al*., 2009). It has recently been estimated that approximately 30% of emerging pathogens identified over the past 60 years can be commonly transmitted by foods (Jones *et al*., 2008). In a report published by

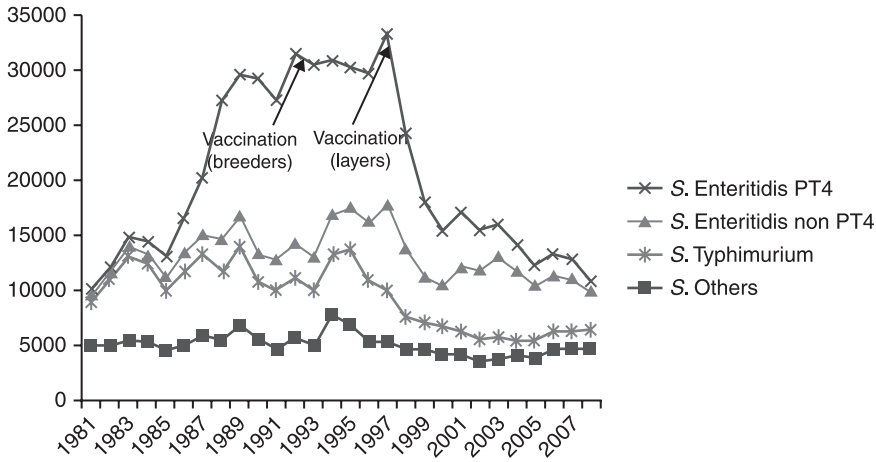

Fig. 2.1 Cases of human salmonellosis by year: England and Wales.

the World Health Organization (WHO) in 2008, the table of leading causes of death worldwide shows that diarrhoeal diseases (the majority of which are food- or waterborne) were the fifth most common cause in 2004 (WHO, 2008a). On a more positive note, the report also states that in children under the age of five, there is some evidence of a substantial decline.

Surveillance of foodborne pathogens is vital in that it provides the opportunity to assess trends in infections, identify new or emerging problems and assess the impact of interventions in a systematic way. This chapter introduces a summary of some of the common surveillance methodologies in use, gives some examples of their use in practise on both the national and international level, and some of the public health information systems and activities in place across the globe. It also looks at some developments for the future and areas whereby the surveillance of foodborne pathogens could be improved. It does not go into every single epidemiological method that can be employed but concentrates on those most applicable to the surveillance of foodborne pathogens.

2.2 Methods for the surveillance of foodborne pathogens

What is clear is that there is not, and cannot be, a single method in use for the surveillance of foodborne diseases. This is the case for other groups of infections, but is more critical for foodborne pathogens due to the very nature of the diverse sources and vehicles of infections involved. Surveillance methods in use range from notification-based, laboratory-based, to sentinel-based systems each with their advantages and disadvantages.

2.2.1 Statutory notification-based systems

These are usually legally binding systems whereby public health practitioners (often general practitioners) have an obligation to provide information to regulatory authorities for a range of notifiable diseases. These diseases are usually defined by national authorities, although some infections are also notifiable under the International Health Regulations (IHR) (see Section 2.3.6).

Advantages: They can provide an early alert of unusual events occurring within the community and will often include basic information that would not necessarily be available to other surveillance systems.

Disadvantages: Notifications are usually based on clinical diagnosis and as such lack laboratory confirmation of the pathogen. In the UK, notifications are made under the category of 'food poisoning' rather than being organism specific. This is defined as 'any disease of an infectious or toxic nature caused by or thought to be caused by the consumption of food or water' (Chief Medical Officer, 1992). There can be some delay in these notifications reaching the national level, which may make it difficult to identify diverse community outbreaks of foodborne infections.

2.2.2 Registration of deaths and hospital discharge data

In the majority of countries when a death occurs, a death certificate is completed. The certificate will include details of the cause of death and basic patient demographics. Similarly, hospitals will report the diagnoses and demographics of patients discharged from their care. When collated centrally, these can provide summaries of the magnitude of specific infections within countries.

Advantages: Registration of deaths and hospital discharge data do provide some data on the burden of illness of infections and can inform studies on the economic costs of these infections.

Disadvantages: These data are mainly acquired for more serious infections and do not represent the morbidity of pathogens in the general community. There is also a tendency to categorise infections under a 'miscellaneous' category rather than being specific, hence will not necessarily capture the full burden of death or hospitalisations for all infections.

2.2.3 Laboratory-based surveillance

Phenotypic methods

Reference laboratory confirmation is considered to be the gold standard of surveillance methods as it gives a precise identification of the pathogen involved. Samples are submitted to a laboratory for testing, and if they are sent to a national reference laboratory, they are then subjected to a range of different tests for characterisation. For bacteria, this would include phenotypic methods such as serotyping, phage typing, where relevant, and antimicrobial resistance testing.

Genotypic methods

The phenotypic characterisation of pathogens is enhanced by genotyping as well. This can be via a range of methods – pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing, amplified fragment length polymorphism, plasmid profiling or multi-locus variable number of tandem repeat (VNTR) analysis – many of which help subdivide phenotypes and enable specific outbreak strains to be identified.

Advantages: Precise identification and characterisation of pathogens allows more accurate ascertainment of foodborne outbreaks of infection. Often outbreaks can only be discriminated from the general background noise by the small, but critical, differences from the routinely circulating pathogen causing many cases of sporadic infection. A small, isolated outbreak of *Salmonella* Typhimurium DT104 within a larger outbreak in the UK in 2000 was only differentiated from the epidemic strain by the identification of a 2.0 MDa plasmid uniquely associated with the smaller outbreak (Horby *et al*., 2003).

Disadvantages: There is inevitably a time delay in the onset of illness and the identification of the causative pathogen (due to time required to submit a specimen to a laboratory and subsequently to a reference laboratory for full characterisation). In addition, it is known that the numbers of cases that are identified in national statistics are only a small subset of people ill in the community. Figure 2.2 shows how the number of cases in the community can be lost between becoming ill and being included in national statistics. As illness resulting from foodborne pathogens tends to present with a mild, self-limiting disease, a large proportion of relatively healthy individuals will not even attend their local physician. Of those who do attend, not all will have a sample taken for testing. Routine diagnostics in the local laboratory may not be able to identify a pathogen, and even when a pathogen is identified, these samples may not be finally submitted to the National Surveillance Institute for inclusion in national statistics. Data from studies in England show that cases of salmonellosis are underreported by a factor of three, campylobacteriosis by a factor of eight and infection with norovirus by a factor of 1500 (Wheeler *et al*., 1999). Similar studies in the Netherlands (de Wit *et al*., 2001) and the USA (Mead *et al*., 1999) have also shown high rates of underreporting, although the multipliers are different due to disparities in referral to primary health care systems and submission to reference laboratories for full characterisation. This loss of data means that the full extent of the burden of illness is often not fully quantified and that epidemiological studies may be hampered by not being able to investigate

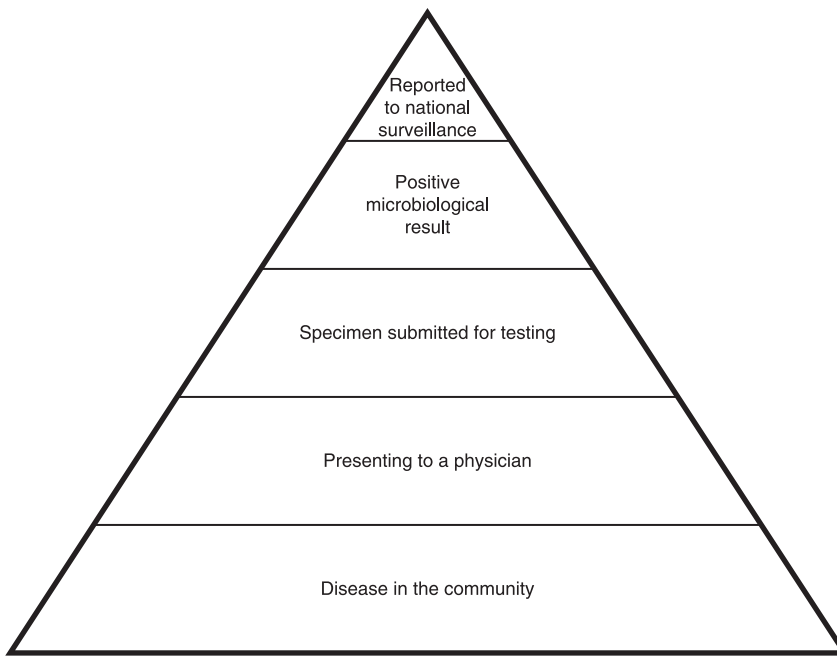

Fig. 2.2 Reporting pyramid with losses of data being reported to national surveillance.

every case. However, this does not prevent outbreaks being identified, successfully investigated and public health interventions implemented.

2.2.4 Sentinel surveillance methods

Sentinel surveillance is conducted on a subset of the national population into a range of infections, not just foodborne. Sentinel surveillance is often used to show the ebb and flow of infections that are usually diagnosed clinically, for example influenza. It can show the spread of infection across continents as epidemics evolve (Arkema *et al*., 2008). In terms of foodborne infections, it can be used as a measure for the incidence of illness in the population and can be tracked across many years. This allows baseline rates to be identified, targets set for reductions to be made and then tracked to determine the success (or failure) of intervention measures to achieve these. It allows for a more in-depth investigation into the risk factors for contracting infections from foodborne pathogens and into the modes of infection.

Advantages: Specific pathogens or syndromes (such as haemolytic uraemic syndrome, an indicator of *E. coli* O157 infection) can be targeted. Risk factors can be identified, subsequent control measures implemented and outcomes evaluated. Denominator data (the population within the sentinel surveillance area) can relatively easily be identified so that the rates of infection can be calculated and provide a definitive number to be measured and changes identified. Data from sentinel surveillance activities can also be used to extrapolate the national incidence of disease to provide the full measure of the burden of infections. Sentinel surveillance is also less resource intensive than investigating all sporadic cases of infection but can provide a large part of the data and information that such investigation would supply.

Disadvantages: Although it is less resource intensive, there is still an additional cost in conducting sentinel surveillance. While this can be done in relatively resource-rich countries, it is difficult to achieve in resource-poor countries that may have different public health priorities. Sentinel surveillance also has to strike a balance between being large enough to allow meaningful analysis and being representative of the population of a country as a whole to ensure that any extrapolations are as accurate as possible and are valuable in assessing the total burden of infection.

2.2.5 Geographical information systems

Geographical information systems (GIS) can be used to build a baseline of endemic levels of infections within a geographical area (usually national or sub-national) and identify when levels of infection breach those expected for that region and time of year. GIS can help illuminate the correlation between infections and environmental or other exposure factors that lead to that infection such as the proximity to sheep and cases of cryptosporidiosis. Mapping cases of infection is a

powerful tool in analysing spatial and temporal trends and is of great relevance to outbreak investigation and control.

Advantages: These can provide evidence of levels of infection above that which is the expected amount and hence assist in identifying outbreaks or clusters of infection to inform investigations into sources of infection. GIS provides a visual reference to investigators and policy-makers that is easily understandable.

Disadvantages: GIS has a tendency to be limited to characterised phenotypes of infection and hence is dependent on laboratory-confirmed cases with the associated time delays. There is also a loss of data as only those cases for which a geographical reference can be identified can be included. This information can often be limited in the case of laboratory-confirmed diagnoses.

2.2.6 Enhanced surveillance for specific pathogens

Enhanced surveillance is valuable for infections with low numbers of cases, but where high public health impacts are involved (e.g. *Listeria monocytogenes*, shiga toxin-producing *E. coli*). Typically, once a case has been identified, either microbiologically or clinically presumptive, questionnaires are sent to patients or their carers to be completed. These ask about the consumption of various foods or possible environmental exposures that may have caused the infection. Results are collated into databases that can be analysed to provide information that would not be available in routine surveillance systems.

Advantages: Enhanced surveillance does provide more in-depth information than is readily accessible normally and hence can provide a greater insight into the exposures that have caused infection. This can be of particular value when different subtypes of bacteria inhabit different ecological niches and may require the provision of different public health messages. Such messages inform the population at risk of potential threats and enable informed action to be undertaken to lessen any possible infection.

Disadvantages: This is a resource-intensive method of gathering information. However, the information gathered and available for analysis can be useful in identifying specific risk factors that can help inform more precise interventions to prevent cases from occurring.

2.2.7 Outbreak investigations

Investigations into outbreaks of infections provide information that is invaluable in both the short term and long term. In the short term, the implementation of control measures to prevent the current outbreak is vital. It is important in outbreak investigations to try and ascertain information about the setting of outbreaks, the demographic details of those being taken ill (which can also provide valuable

information on the vehicle of infection), what was the pathogen involved and what were the contributory factors that allowed the outbreak to occur. Evidence gathered from the investigation of outbreaks in the longer term help inform policy-makers of potential strategic interventions than can be implemented in the future.

Advantages: Full-scale epidemiological and microbiological investigations into all outbreaks may not be necessary on every occasion, but they can be beneficial in identifying new vehicles and modes of transmission that may not have been recognised before. The early part of the twenty-first century has seen a substantial increase in the number of outbreaks associated with salad products (Grant *et al*., 2008; Greene *et al*., 2008; Horby *et al*., 2003; Wendel *et al*., 2009). These are ready-to-eat products that are not intended for further heat treatment prior to consumption. If they do become contaminated during harvesting, then very few opportunities for a reduction in levels of pathogens on the food exist before being eaten. Outbreak investigations can also provide valuable information into microbial risk assessment, dose–response relationship and the ecology of foodborne pathogens.

Disadvantages: Outbreak investigations are resource intensive, and the benefits of investigating every outbreak may outweigh the cost of doing so. In addition, investigations can be hampered by being detected too late, allowing recall bias to influence the results of any study. There may not be any foodstuffs available for microbiological testing to be performed to confirm the pathogen in the food as well as in human cases involved in the outbreak, or an agent may not be identified at all. An example of the latter would be foodborne outbreaks associated with viruses that can be difficult to confirm, particularly in foods. Not all outbreak epidemiological studies will definitively identify a foodstuff associated with illness. This may be due to more than one food being contaminated, not asking the right questions in an investigation or an outbreak being too small to achieve a statistical significance. Nevertheless, such investigations are always useful as lessons can be learnt from even the most straightforward outbreaks.

2.2.8 New methods for surveillance of epidemic intelligence

New information systems based on the gathering of ‘epidemic intelligence’ (Paquet *et al*., 2006) are becoming more common in collating information on events being identified by the media and on the Internet, thus bringing them to the attention of public health officials. These systems can be valuable in identifying events before they become formally known. Official systems currently in place include GPHIN (Public Health Agency of Canada, 2004), MEDYSIS and EPIS. GPHIN is the Global Public Health Information Network run by the Public Health Agency of Canada on behalf of the WHO. It trawls media sources across the globe and in multiple languages to identify events involving infectious diseases and circulates them to the public health community. MEDYSIS

(European Commission, n.d.) and EPIS, the Epidemiological Information System, are similar tools that have been developed by the European Commission and the European Centre for Disease Prevention and Control (ECDC), respectively. GPHIN, MEDISYS and EPIS are restricted access sites, although since December 2006, MEDISYS has also had a public domain version.

In addition to the above restricted or limited access sites, there are similar non-governmental electronic reporting systems such as ProMed-mail, which is the Program for Monitoring Emerging Diseases and is a program of the International Society for Infectious Diseases (ProMed-mail, n.d.) and the International Food Safety Network, which has been succeeded by Bites – Safe Food from Farm to Fork (Kansas State University, n.d.). Both of these systems either trawl public domain web pages or allow information on infectious disease to be posted on them. This information is then circulated (usually via email) to anyone who has subscribed to their distribution lists.

Advantages: The media (especially the Internet) is often the first to hear the instances of foodborne, or potentially foodborne, outbreaks of infection, and they often can be the first source of information received by public health agencies.

Disadvantages: The sheer volume of noise can make a full assessment of every report that may be an outbreak impossible to achieve. However, this useful source of information should not be ignored and can be used to complement information received from official sources.

2.3 National and international surveillance systems in use

Most countries have systems in place for collating data on notifiable diseases and registrations of deaths plus hospital discharge information along with phenotypic laboratory-based surveillance. These are successful to a greater or lesser extent depending on the public health infrastructure and commitment within each country. They are of value in highlighting unusual events that may be indicative of potential foodborne outbreaks of infection or in identifying deficiencies in food production practises that require measures to improve them. Much work remains to bring all countries up to a set standard. As all countries will benefit, this has to be a priority for public health in the future. The WHO is attempting to address these limitations by defining the set of 'core competencies' (see Section 2.3.7) that will set the baseline standards for the future.

2.3.1 Notifications, death registrations and hospital discharge data

Data on disease notifications, death registrations and hospital discharge are collated and published by national governments on a regular basis. They are not highly specific to particular foodborne pathogens but do provide assessments of the relative burden of these, particularly at the severe end of the spectrum.

2.3.2 Phenotypic and molecular typing networks – Global Salm-Surv, PulseNet US and PulseNet International

Phenotypic methods for typing foodborne pathogens have been an integral part of the identification of foodborne pathogens for many years, and as such have been invaluable in identifying causative agents and their sources. This has provided information to enable both short-term and long-term intervention measures to be put in place by policy-makers and industry. Global Salm-Surv is a WHO-led initiative that promotes capacity building in the detection, control and prevention of foodborne pathogens (Galanis *et al*., 2006). Global Salm-Surv has been superseded by the Global Foodborne Infections Network. In the recent years, genotypic methods have come to the forefront. It has been stated that molecular typing has transformed public health by providing methods to further discriminate pathogens and identify clusters of cases that may not have been possible previously (Tauxe, 2006). This is certainly the case. The acknowledged method of choice in DNA-based methods has been that of PFGE to differentiate strains of bacterial pathogens. Standardised/harmonised PFGE methodology was initiated formally in the USA (Swaminathan *et al*., 2001) and has subsequently been expanded to include other countries across the globe (Swaminathan *et al*., 2006). PulseNet International brings together the six regional PulseNet networks (Asia-Pacific, Canada, Europe, Latin America and the Caribbean and the USA) into one microbiological network. PFGE is not the only method in use but is the most common. An advantage of using harmonised methodology such as the PulseNet system is that electronic images of strains can be compared directly without having to transfer strains to reference laboratories for characterisation. This negates the resulting additional cost involved in paying for the shipment of strains and inevitable time delay in getting them to their destination. An integral part of any such system has to be quality assurance to ensure that the right outputs are provided across laboratories. The key to creating successful laboratory networks that are able to identify and compare strains is by using validated methods for subtyping. PFGE, as described, has been the cornerstone of international public health microbiology, but new methods are being developed that can also be applied internationally. VNTR is rapidly becoming a method of choice for some *Salmonella* serovars, although currently only protocols for *S.* Typhimurium have been validated and published (Lindstedt *et al*., 2004). Considerable work is currently being undertaken to develop VNTR techniques for other serovars (Larsson *et al*., 2009) and other pathogens such as *E. coli*.

2.3.3 Sentinel surveillance

In the area of foodborne pathogens, the most obvious examples of sentinel surveillance are that of FoodNet in the USA and OzFoodNet in Australia (Kirk, McKay, *et al*., 2008). The most recent FoodNet report gives preliminary details of the incidence of laboratory-confirmed bacterial and parasitic infections in ten participating states in the USA (CDC, 2009). It shows for a range of infections under surveillance the incidences of each of these in 2008 compared to the targets