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REVIEW ARTICLE

Does microbiological testing of foods and the food environment have a role in the control of foodborne disease in England and Wales?

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Summary

This review looks at the contribution of microbiological sampling to the safety of retail foods in England and Wales. It compares sampling methods available and assesses the value of testing as part of outbreaks of foodborne disease, as part of routine management by local authorities, as part of work done or commissioned by the food industry, and as part of research. It confirms that microbiological testing has a role during outbreaks as it makes a significant contribution to help identify foods and other areas of greatest risk for future study. The review suggests that routine testing by local authorities is often of limited use and could be improved by more targeted surveillance. Testing could be better used to validate primary control methods, such as Hazard Analysis and Critical Control Point (HACCP) system. Any public health benefit from testing in the food industry is often restricted by client confidentiality. Microbial research on foods is important as it can lead to significant improvements in safety. Current microbiological methods are slow and, in future, rapid molecular methods may make an even bigger contribution to the control of foodborne disease.

Introduction

Although the actual incidence of foodborne disease is unknown, most experts agree that current levels are unacceptably high. Using data from population-based studies and national surveillance collected between 1996 and 2000, one study estimated that more than 1·7 million cases of indigenous foodborne disease occurred in the UK per year (Adak *et al.* 2005). Whilst most cases are self-limiting, some, particularly in the very young or old and those with underlying disease, can be serious and even life-threatening (DuPont 1997). The burden on health-care systems is considerable (Frenzen *et al.* 1999) and the cost to the NHS and business was estimated as £350 million per year by the Food Standards Agency (FSA) in 2000. Although control of foodborne disease is a top priority of the FSA (FSA 2000), evidence shows that most cases go unreported (Wheeler *et al.* 1999).

All authorities in the UK now follow a risk-based approach and a Food Law Code of Practice provides instructions to officers responsible for inspections (FSA 2004). Member states of the EU are required under article 14 of the Official Control of Foodstuffs Directive 89/397 to submit annual returns on official food enforcement activities. In the UK, this information, including microbiological sampling results, is collected from local authorities by the FSA (FSA 2005). The process plays an integral part in each authority's food surveillance programme. Its prime aim is to detect food contaminants, be they microbiological, chemical or physical, before they cause a risk to public health. Although some variation in food sampling rates is inevitable, the FSA has criticised some authorities for not achieving adequate levels of sampling and therefore putting public safety at risk.

In a recent survey, Wong *et al.* (2004) tried to measure the effectiveness of food sampling by local authorities

using a questionnaire sent to each authority. Of the 40% of authorities that replied, about half who gave any opinion thought their programme did not contribute to the prevention of foodborne disease. On the basis of responses received, the authors questioned the effectiveness of the current food sampling programmes. Over three-quarters of the local authority staff who responded thought the system could be improved, particularly by increasing resources to allow more sampling.

The purpose of this review is to consider the role of testing for micro-organisms, or specified parts of them, in foods and the food environment. The review first compares the sampling methods available, then examines the different locations in which sampling is done, and finally discusses what sampling is of benefit and whether or not the current strategy achieves its purpose.

Test methods

Foods

Traditionally pathogenic bacteria from suspect foods are isolated by agar culture either after pre-enrichment to help the recovery of any damaged organisms and selective enrichment promoting the growth of the target organism or by direct quantitative culture on a specified medium (Roberts and Greenwood 2003). This is a slow but generally reliable process. Although a small number of pathogens account for the vast majority of foodborne illnesses (see Adak *et al.* 2002), new pathogens are emerging and known pathogens may be found in food types not previously associated with them (Tauxe 1997). Good examples include the recent discovery of *Enterobacter sakazakii* as an important pathogen in powdered infant formula (Drudy *et al.* 2006) and the increased risk from globalisation of fresh produce, e.g. uncooked fruit and vegetables (Sivapalasingam *et al.* 2004).

There has been a lot of debate over the existence of non-culturable forms of bacteria and whether these contribute to foodborne illness (Murphy *et al.* 2006). Certainly, bacteria in foods may be stressed, for example, by exposure to heat or cold; however, these organisms can often be recovered after appropriate enrichment. Although a significant proportion of bacteria are not culturable, and some have been detected in foods by molecular techniques, their significance as foodborne pathogens is unknown.

The food environment

Foods may be contaminated from the processing environment, and testing at this level can act as an early warning system with the risks being identified and controlled before pathogens enter the food itself (Tompkin 2004). Environmental contamination can come from water, air, food-contact surfaces and personnel (Holah 1999). Direct airborne contamination of foods by pathogens is uncommon but has been recorded (Varma *et al.* 2003; Drudy *et al.* 2006). Air sampling might be considered in an outbreak if the cleaning methods in use were suspected of generating aerosols (Gudbjornsdottir *et al.* 2004).

In most cases, process water is obtained from a water supply company that treats water to kill harmful micro-organisms, and so it poses little risk unless stored incorrectly and allowed to become contaminated. The main risk is from untreated or inadequately treated water supplies (Duffy 1999; Said *et al.* 2003). Spraying crops with contaminated water could also lead to contaminated food (Carter 2005). Water in domestic washing-up bowls was considered to be a possible vehicle for both *Salmonellas* and *Campylobacters* as the water temperature was generally lower than that needed for adequate cleaning (Mattick *et al.* 2003).

Sampling food-contact surfaces can be done by swabbing (Tebbutt 1986, 1988; Tebbutt and Southwell 1989, 1997; Humphrey *et al.* 1994; Snyder 1997), by sponges (Sagoo *et al.* 2003a; Thimothe *et al.* 2004) or by contact or replica plating (Scott and Bloomfield 1990; Tebbutt 1991a,b). Sponges are preferred for sampling a large area, such as the whole of a cutting board or the surfaces of several boards, in the search for pathogens. Conversely, looking for pathogens in food debris, which has built up between joints in surfaces, is better done by swabbing to tease out material from gaps and crevices. Sampling reusable cloths is usually done by submitting the whole cloth to the laboratory either in sterile bags (Sagoo *et al.* 2003a) or placing in bags and adding a suitable neutraliser solution for any disinfectant present (Tebbutt 1991a,b). Non-contact surfaces such as floors, ceilings and drains are not likely to contaminate the product directly; however, these can be reservoirs and organisms can be aerosolised from them by cleaning systems. Although the best surfaces for sampling partly depend on the circumstances of investigation and what is available, those tested should reflect observed or perceived risks in the premises.

What parameters should be looked for?

The debate about testing for pathogens or indicators goes on. Lack of consistency restricts the value of many studies. In the UK, national surveys that are co-ordinated by the Health Protection Agency (HPA) and representatives from local authorities partly overcome this by specifying the products to be tested and the parameters to be looked for (see local authority sampling section and Table 1). Although these surveys contribute to a national microbio-

Table 1 Some examples of national surveys on the microbiological quality of ready-to-eat foods with particular reference to the presence of salmonellas, campylobacters and *Listeria monocytogenes*

Food type	Number of samples assayed	Satisfactory (%)*	Number of samples positive for			Reference
			Salmonella	*Campylobacter*	*L. monocytogenes*	
Meats	2192	84	2	0	NE†	Little and de Louvois (1998)
Sliced cold meat	3494	80	0	NE	13/3442	Gillespie *et al.* (2000)
Chicken	534	NE	0/529	0/512	NE	FSAI (2001)
Sliced ham	923	75	0	NE	0/919	FSAI (2005)
Quiche	2513	94	0	NE	NE	Gillespie *et al.* (2001)
Burgers	3128	92	0	0	NE	Little *et al*. (2001)
Organic vegetables	3200	99·5	0	0	0	Sagoo *et al*. (2001)
Chicken	449	75	0	0	NE	Little *et al.* (2002)
Shopping basket	15228	83	1	0/2061	57	Meldrum and Ribeiro (2003)
Various	4469	NE	NE	0	NE	Meldrum and Ribeiro (2003)
Salad vegetables	2950	97	0	0	1	Sagoo *et al*. (2003)
Salad vegetables (bagged)	3852	99	5	NE	1	Sagoo *et al*. (2003)
Cold meats/Pate	4078	75	0	1	1	Elson *et al.* (2004)
Number (%) with pathogen detected			8/40344 (0·02)	1/23039 (0·004)	73/33669 (0·22)	

*Gilbert et al (2000) PHLS Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. Satisfactory is based on the aerobic count at 30°C, the absence or presence at low levels of the indicator bacteria *Enterobacteriaceae* (<100 CFU g^{-1}), *E.coli* (total –<20 CFU g^{-1}), and *Listeria* spp. (total <20 CFU g^{-1}), and the absence of the pathogens tested in a 25 g sample.
†NE, Not Examined.

logical database on foods and their environment, they also highlight the rarity of pathogens in ready-to-eat foods; a result suggesting that routine testing for them is not worthwhile. There is a need to focus on foods that present the highest risk. Eggs, particularly those imported into the UK, have been linked to a greater risk of acquiring salmonellosis, and increased sampling frequency has contributed to a reduction in this risk. In most UK surveys unit samples are tested; however, several sample units are examined from a lot as part of international trade (Dahms 2003). This is important as bacteria are not evenly distributed in foods, and testing a representative sample is very difficult.

Indicator organisms can play a part in validating Hazard Analysis and Critical Control Point (HACCP) or other food safety management systems. Their presence can point to processing failure, poor hygiene practice and the potential for contamination by pathogens. Given that indicator organisms occur more often than pathogens, their presence is not a reliable index that a pathogen is present (Kornacki and Johnson 2001). Jay (2000) reviewed the use of bacterial indicators of food safety, including *Escherichia coli, Enterobacteriaceae* and enterococci, and emphasised the need to choose the correct indicator for the type of product being assessed. Busta *et al.* (2003) compared indicator and surrogate organisms in fresh or fresh-cut fruit or vegetables. Friedhoff *et al.* (2005) considered that *Enterobacteriaceae* counts together with aerobic colony counts provided adequate information to verify a HACCP system. A count above a specific level would suggest increased risk of enteric bacterial pathogens; however, the association is far from perfect and is clearly inappropriate for viruses and preformed toxins. Some experts consider bacteriophages as indicators of the presence of harmful viruses (Allwood *et al.* 2004). In future, molecular identification of genetic material common to indicators and pathogens may provide the way forward.

A majority of cases of foodborne disease in the United States are caused by noroviruses (Payne *et al.* 2005; Widdowson *et al.* 2005). This virus accounts for the vast majority of non-bacterial outbreaks of gastroenteritis. Although estimates of foodborne transmission vary among countries, about 7% were thought to be transmitted by this route in England and Wales (Lopman *et al.* 2003). Although tests for the detection of virus in foods or the food environment are not widely available, foods that are handled during preparation and not heated prior to consumption are at greatest risk (Widdowson *et al.* 2005), and there is a risk of virus transfer from hands (Payne *et al.* 2006) and environmental surfaces (D'Souza *et al.* 2006). Food studies are hampered by the inability to grow norovirus in tissue culture; however, a study with

a surrogate (Feline calicivirus) in oysters suggests that viruses are more heat resistant and more difficult to remove by depuration than *E. coli* (see Sair *et al.* 2002 for review).

Sampling as part of suspected outbreaks

Guidance on the investigation and control of outbreaks has been published and updated by the FSA (see FSA 2006). A microbiologist, as a part of the Outbreak Control Team, gives advice on appropriate clinical specimens as well as the testing of foods and the food environment. However, no pathogen has been recovered in a number of suspected foodborne outbreaks.

Establishing a specific link between the source of contamination and illness is an important part in outbreak investigation. Finding an organism that is indistinguishable from the human isolates, in prepared foods or in the processing environment, helps confirm a link with the source. Adak *et al.* (2005) reviewed the results of food testing in a large number of outbreaks, noting the significance of different food types. Chicken was consistently responsible for more infections identified between 1996 and 2000. Eggs, egg products and the use of raw shell egg have been significantly linked to foodborne outbreaks of *Salmonella enterica* serovar Enteritidis phage type 4 infections (Gillespie *et al.* 2005). Other *Salmonellas*, notably *S.* Enteritidis PT14b, have also been linked to outbreaks from eggs imported into the UK (Holtby *et al.* 2006). Holtby *et al.* (1997) reported the isolation *S.* Enteritidis PT6 from several environmental sites including a reusable cleaning cloth in an outbreak caused by this *Salmonella*.

Although positive laboratory results support outbreak investigations, successful legal action is not actually dependent on them. A restaurant owner was held responsible for an outbreak in 2003, caused by *S.* Enteritidis PT56 (a very rare strain) affecting more than 300 people, pleaded guilty in the face of overwhelming statistical and epidemiological evidence (Clapham *et al.* 2006). Nonetheless, most local authority officers consider positive laboratory evidence as good evidence readily understood in court. That is not to say that the evidence may not be challenged, and it must follow established procedures for sampling and transportation with standard test methods being used in a laboratory.

When food poisoning is suspected, obtaining the suspect food is of primary concern to the investigating officer. Sometimes, small quantities, perhaps left-over food taken home after a function and retrieved from a waste bin, are all that is available. Although the continuity of evidence is lost, these samples can be important in outbreak investigations (Holtby, personnal communication). Obtaining outbreak-associated food samples for laboratory testing is often based on the results of epidemiologic case-control studies. If food samples are not taken, there is nothing wrong with collecting a number of items for refrigerated storage and testing at a later date. The storage temperature needs to be carefully monitored. Freezing is detrimental to some bacteria, but may be necessary for storage longer than a few days.

Sampling the food-processing environment is important, and prior cleaning should not preclude this being done. Cleaning can sometimes spread contamination across the kitchen. Reusable wiping cloths can transfer bacteria to foods via surfaces and hands (Gilbert 1969: Tebbutt, 1991a,b). Not all relevant sites are included in cleaning programmes, and some, such as refrigerator door handles, are touched frequently when preparing foods (Tebbutt *et al.* 2007). The risk is further increased if both raw and cooked products are kept in the same refrigerator. Some sites are inaccessible to all but the deepest cleaning, and pathogens may remain in these areas for some time. In one outbreak, the detection of a *Salmonella* indistinguishable from the outbreak strain was found in a poor joint between work surface and sink even after thorough cleaning had been carried out by staff at the premises (Holtby *et al.* 1997).

One of the main problems that limit sample collection is late notification of a possible outbreak. Many people do not consult a doctor, and sometimes many doctors do not suggest submitting the faeces for testing, citing that the illness is self-limiting and doing so would burden the local laboratory. This makes investigation more difficult and leads to potentially valuable evidence from food and environmental testing being lost.

Sampling by local authorities as part of food safety programmes

Government funding is provided to enable microbiological sampling to be carried out by local authorities. Currently, laboratories belonging to or commissioned by the HPA examine almost 100 000 food, water, dairy and environmental samples each year with over half of them being foods. Large national surveys allow statistically significant trends to be identified with the test protocols being standardised and the results analysed centrally. Table 1 shows the results of some national food surveys and illustrates how infrequently pathogens such as *Salmonella* (0·02%), *Campylobacter* (0·004%) and *Listeria monocytogenes* (0·22%) are found in foods offered for sale. When the incidence of pathogens is extremely low, routine testing may no longer promote food safety.

Various attempts have been made to link visual inspections and the results from microbiological testing.

Tebbutt and Southwell (1989) studied local food manufacturers and found no association between microbiology and the overall appearance of the premises, personal hygiene practices, risk of contamination of final products, temperature control during cooking, and the training and education of staff. Powell and Attwell (1995) questioned the value of inspections and found no correlation between visual inspection ratings in retail premises and the microbiological quality of food samples tested. Jones *et al.* (2004) reviewed the data of inspections on restaurants in one state of USA between 1993 and 2000. They found that the mean scores of restaurants that had been involved in a foodborne outbreak were not different from those with no reported outbreak. Conversely, Irwin *et al.* (1989) reported that both poor inspection scores and unsatisfactory temperature records were related to an increased risk of foodborne outbreaks. Little *et al.* (2003) reported a meta-analysis of eight UK national food studies using the PHLS Microbiological Guidelines (Gilbert *et al.* 2000) and found a significant association between good microbiological results and the operation of HACCP training by retailers and caterers.

Sampling by the food industry

Much of the information in this area is retained within the industry and not made public. Microbiological testing often provides quality checks as part of processing. One aspect that producers are well aware of is the use of testing to provide a defence of due diligence as operators have a duty to withdraw unsafe food (European Commission 2005). Although no amount of testing, at least up to the point that makes economic sense, can guarantee safety, being able to demonstrate that all reasonable steps were taken is a good defence in a court of law.

Sampling by research organisations

Although microbiological testing of foods is frequently commissioned by the food industry, the publication of results may be restricted by client confidentiality. Roberts (1997) reviewed the usefulness of food microbiology research, concluding that better project design and wider availability of results were needed. Funding may be made available after disease outbreaks for fundamental research such as understanding how the pathogenicity mechanisms of organisms work or studying the ways in which potential pathogens interact with resident gut flora. The shelf life of trial products to which less or new preservatives have been added may be investigated by challenge and other testing. The desire for minimally processed foods and natural food products without preservatives is the driving force behind a number of products. New products, particularly if they involve novel processes or technologies, may need extensive microbiological testing prior to marketing.

Sampling that does not require the isolation of bacteria

Microbial sampling is slow and often retrospective unless food is held subject to positive release. Some molecular tests are available. These may save time but, to match the sensitivity of conventional culture, they still depend on enrichment and cannot be classified as truly rapid methods. A key obstacle to routine use is the need for standard and accredited test procedures that will stand up to scrutiny in a court of law. Real-time genetic-based assays are being developed and should be able to detect very small amounts of target DNA in foods (Fratamico and Bayles 2005; Naravaneni and Jamil 2005). Comparison between these molecular methods and culture is generally good, with newer methods performing at least as well and sometimes better (Bohaychuk *et al.* 2005; Lin *et al.* 2006). Next generation assays including biosensors (Anderson and Taitt 2005; Rasooly and Herold 2006) could be both rapid and cost-effective.

Poor cleaning leaves behind food debris on food-contact surfaces and equipment. Although not detectable by microbial sampling, the debris is important as it may allow the multiplication of small numbers of bacteria left on the surface. Detection of ATP (a substance present in all living cells) can be used to monitor cleaning efficiency. The method is widely used in the food industry (Stannard and Gibbs 1986; Holah 1999) and has been used to monitor cleaning standards in a hospital kitchen (Tebbutt and Midwood 1990). Recently, ATP detection was included as part of inspections of food premises by local authority officers (Tebbutt *et al.* 2007). The use of protein left behind on surfaces after cleaning can be used, and this parameter has been compared to ATP and microbiological sampling (Moore and Griffith 2002). Both ATP and protein tests are simple to use, rapid and, given that visual assessment is a poor indicator of surface cleanliness, are valuable tools for assessing cleaning practices (Powell and Attwell 1997; Griffith *et al.* 2003).

A significant amount of work has been done on predictive modelling with the introduction of interactive models under a range of conditions and variables (McMeekin *et al.* 1997). The models not only analyse the behaviour of pathogens in food but can be used to predict the growth of spoilage organisms and their effects on shelf life. Microbiological sampling plays a part in establishing these models and in validating and verifying their performance by challenge testing.

Testing and food safety

How testing contributes to food safety?

In 1998, it was estimated that some 400 million food tests were performed across the world (see Fung 2002). A majority were quality tests carried out in food laboratories. Nevertheless, 10–20% of tests were done to look for pathogens, and these could be considered attempts to promote product safety. A recent evaluation of the foodborne disease strategy of the FSA (Bell 2006) looked at various strategies, campaigns and interventions put forward by the FSA. Although local authority sampling programmes were considered important as part of this strategy, no evidence was presented to link the programmes with a reduction in food safety risk. It is a policy for authorities to revisit premises with poor microbiological results, usually high aerobic counts, but there is no direct association between high counts and safety, and benefits from this strategy are not verified.

To complement the new food hygiene legislation introduced in the UK from 1 January 2006, the FSA has set out microbiological criteria for various foodstuffs. Although these can help to validate and verify food safety management systems, the regulation does not advocate routine end-product testing. While the regulation seeks to reduce contamination of foods, it is far from clear how it will be regulated nationally and whether or not periodic testing, as envisaged, will add anything to an effective HACCP programme. The FSA proposes to review the guidelines after 12 months.

End-product testing, particularly if the rate of contamination by pathogens is very low as would be expected in processed foods, cannot by itself protect the consumer. Unless impractically large numbers of products are examined, testing is statistically invalid and potentially misleading. It may be argued that a significant amount of food testing carried out by local authorities falls into this category, even if we accept that this sampling may occasionally throw up a pathogen by chance. Environmental sampling is a useful tool to verify the effectiveness of cleaning measures, a process that cannot be done by observation alone. Rapid techniques, such as ATP testing, provide for real-time interventions. Although ATP measurements will detect microbes, the main contribution is from foods being processed. Some foods have low ATP levels, and with these the value of testing diminishes. If only microbial ATP was present, the bacterial load must be very high before testing would highlight a problem. Rapid tests are used by the food industry to provide local authority officers with on-the-spot evidence as part of inspections. They should not be used alone as part of outbreaks where the detection of the pathogen is desirable so that epidemiological typing of isolates can be performed.

Which sampling is not effective?

There is no public health benefit from repeated testing of products or premises unless an ongoing risk has been identified or is strongly suspected. Sampling needs to be targeted on those foods or premises that present the highest risk. Results need to be tabulated, made available to others, and need to be acted upon. Too often, sampling continues in the belief that good results are the reason for safe products.

Conclusions

i) There is a need to expand current foodborne surveillance. In suspected outbreaks, microbiological testing is important both to identify foods that are more likely to be associated with disease and to pinpoint areas where controls have broken down. This allows future work to concentrate on areas that will have the greatest impact on safety.

ii) Current microbial testing of routine foods may be driven by the need to show that something has been done. Sampling by local authorities is neither standardised nor evenly applied across England, and positive results for pathogens appear to occur more by chance than good planning. Routine testing might do more good if it is linked to the programme for inspecting food businesses on the basis that observation alone may fail to identify some bad practices. Any surveys should be large enough so that the results can be properly analysed and should concentrate on high-risk foods or practices identified by foodborne disease surveillance programmes. The latter need to be increased above current levels and the findings need to be widely circulated.

iii) Databases on pathogen contamination of foods and the environment are needed. Effective communication both between agencies concerned with public health and within the food industry is important. Information at present held within the industry needs to be opened up, perhaps with the creation of an anonymous database for microbiological results.

iv) Close co-operation between laboratory staff and both local authority officers and communicable disease physicians is vital. The laboratory service is not just about providing test results, and senior laboratory staff should be encouraged to provide expert microbiological advice on site. In future, rapid molecular testing will better support investigations with not only a range of pathogens being sought in the same test but fingerprinting the causative agent simultaneously.

v) Without doubt, hazard analysis programmes and microbiological risk assessments provide the most effective solutions to reducing foodborne disease in the commercial sector. However, given the right planning, microbiological sampling has a role to play both by highlighting and monitoring foods and practices that have the greatest risk and by validating procedures that cannot be judged by observation alone.

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