Microbial update

Salmonella

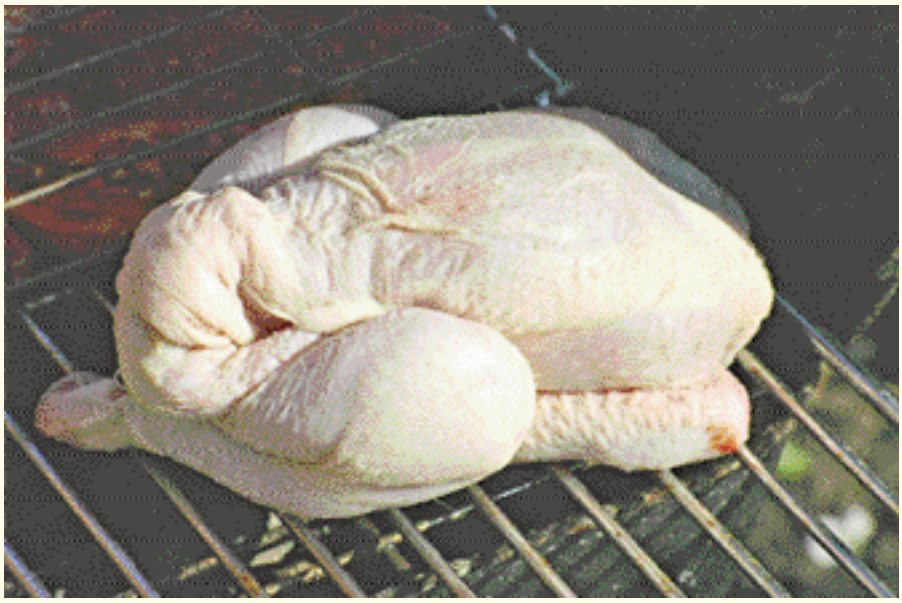
The incidence of salmonella food poisoning declined in Europe by 9.5% in 2005, being overtaken by campylobacteriosis as the most reported animal infection transmitted to humans in the European Union.

However, with a reported incidence rate of 38.2 per 100,000 people in the EU, Norway, Iceland and Switzerland, prevention of salmonellosis remains a serious public health challenge. Inevitably, food manufacturers, processors and retailers continue to find themselves on the front line in the battle to prevent salmonella contaminated foods endangering the health of consumers. Meanwhile, the range of foods – both animal and vegetable – being implicated in product recalls and actual outbreaks of salmonellosis grows ever wider.

Spinach, mung bean and alfalfa sprouts, tomatoes, chocolate, tea and cantaloupe melons all found their way on to the list of products variously recalled from supermarket shelves around the world in 2006, having tested positive for salmonella. They take their place alongside the raw meats, poultry, eggs and dairy products more commonly associated with the pathogen.

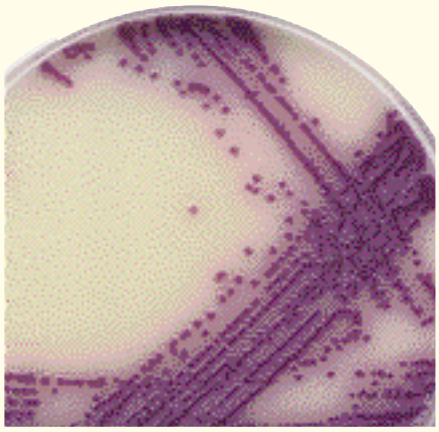
The World Health Organization regards salmonellosis as a major public health burden, citing an estimated total cost associated with salmonella infections in the United States of US$ 3 billion per annum. This estimate includes medical costs due to illness, the cost of time lost from work due to non-fatal illness, and the cost of premature death. The US is one of only a few countries worldwide to report data on the economic cost of the disease: another is Denmark, where the annual estimated cost of food-borne salmonellosis represents approximately 0.009% of Gross Domestic Product (GDP). Such cost estimates, however, take no account of the human suffering associated with salmonella infections (diarrhoea, vomiting, fever and sometimes death), nor of the substantial damage that can be done to the food companies involved in terms of litigation costs and tarnished reputations.

Thus, the importance of reliably detecting the presence of salmonella in foods prior to their distribution and consumption cannot be underestimated.



Raw chicken is commonly associated with the salmonella pathogen (Shutterstock).

Oxoid Salmonella Chromogenic Media Mark II.



New challenges

Whilst the range of foods in which salmonella is being found appears to be widening, eggs and poultry continue to provide a major focus for intervention programmes designed to prevent the pathogen passing along the farm-to-fork chain.

A recent report from the European Food Safety Authority (EFSA) shows a high, linear correlation between the prevalence of salmonella in laying hens and the incidence of salmonellosis, while the US Center for Disease Control (CDC) reports a four fold increase in positive test results for Salmonella enteritidis on chicken carcases, a strain that had previously been associated mainly with eggs.

Another growing challenge is that of the emergence of strains of salmonella which are resistant to a range of commonly used antibiotics – a trend which has been evident since the 1990s. This resistance appears to result from the use of antimicrobials both in humans and animal husbandry. Multi-drug resistance to 'critically important antimicrobials' is compounding the problem.

So, despite an encouraging fall in the overall incidence of human salmonellosis, it is clear that food industry laboratories must continue to place the highest importance on ensuring that products remain salmonella free.

The complexities of culture

Detection of salmonella by traditional methods is dependent on successful isolation of the pathogen using appropriate culture media. The choice of 'appropriate' media is influenced by the nature of the food under investigation and the characteristics of competing micro-organisms. There is no single 'correct' methodology that can be applied to all foods and all circumstances.

The methods and media employed must be capable of enabling growth to occur from extremely low initial cell numbers, bearing in mind that these cells may be stressed or have sustained damage during the processing of the food. It follows that the media in use should initially be capable of resuscitating salmonella prior to providing them with a selective advantage.

Satisfactory resuscitation and pre-enrichment generally requires use of a non-selective medium such as Buffered Peptone Water or Lactose Broth. However, these general purpose media may be unsuitable for use with samples which contain a high population of lactose fermenting or Gram positive organisms, in which case a more specific pre-enrichment medium must be chosen. Examples of these are distilled water with added brilliant green dye for testing dried whole milk and Tryptone Soya Broth for herbs, spices, dried yeast and egg products.

Selective enrichment broths are employed for the purpose of increasing the salmonella population whilst at the same time inhibiting multiplication of other organisms in the food sample. Bile, tetrathionate and sodium selenite are among the variety of inhibitors commonly used in such broths, along with the dyes brilliant green or malachite green and antibiotics such as Novobiocin. The activity of these inhibitory agents may be further enhanced by incubation of the enrichment culture at temperatures greater than 37°C.

A similar range of selective agents is also found in the plating media used for culture of salmonella. It must be borne in mind that not all serovars of salmonella will grow equally well on all plating media, and the success with which individual media suppress contaminating flora also differs. The actual choice of medium, where this is not strictly laid down by a specific protocol, should take account of the food under investigation and the competing flora that is likely to be present in the selective enrichment broth.

Suspect colonies which grow on plating media must be sub-cultured to a non-selective medium such as nutrient agar or broth, from which colonies or a suspension can be taken for confirmatory testing. The results from these reactions will build on the diagnostic information already obtained from culturing.

The development of chromogenic media such as those in Oxoid's Brilliance range facilitate easy identification of suspect colonies by means of vivid colour reactions.

Oxoid Chromogenic Salmonella Medium Mark II (OSCM II) is the first such medium to incorporate a unique new class of selective agents known as Inhibigens which, when added to a culture medium, provide highly specific selectivity and allow improved recovery of stressed salmonellae.

New rapid methods

Traditional culture methods require three to four days to obtain a presumptive positive result. Such lead times are undesirable when testing products with relatively short shelf lives and this has given rise to the development of various innovative methodologies. For example, the Oxoid Salmonella Rapid Test combines enrichment, selective growth and presumptive identification of motile salmonella species in a single culture vessel, with results available in less than half the time taken by traditional culture methods.

One of the latest developments in culture technology is a rapid culture method which facilitates detection and differentiation of salmonella from food samples in less than two days. This method combines the use of a single enrichment broth plus chromogenic plating medium: One Broth Salmonella, a nutritious medium for the recovery and growth of salmonellae which also inhibits the growth of competing organisms and allows recovery of stressed salmonella cells – even when present in very low numbers. Rapid identification and differentiation is then achieved using OSCM II.

Various rapid methods designed to deliver presumptive results within a matter of minutes or hours at the post-enrichment stage are also available. These include ELISA methods, lateral flow test strips which can be used to detect the presence of salmonella cells in either food or environmental samples, and immunomagnetic separation beads can also be used for rapid selective concentration of salmonella from pre-enriched samples.

To assist confirmation of presumptive salmonellae, latex agglutination kits, miniaturised biochemical tests (such as Oxoid's Microbact and OBIS Salmonella) are available.

Of course, the advent of molecular diagnostic technology has opened up important alternative avenues in respect of salmonella detection. Use of DNA based polymerase chain reaction (PCR) methods allows food laboratories to detect and enumerate specific pathogens in hours rather than days, working either direct or from enrichment of raw ingredients, finished products and environmental samples. The automated DuPont Qualicon BAX system, (distributed in Europe, Australia and Canada by Oxoid) has been adopted by many food industry manufacturers and processors worldwide as the method of choice for detecting salmonella in their products.

For food manufacturers and processors, time is of course very much of the essence when screening for the presence of salmonella. New developments now mean that shortened test time need not compromise the quality of result. n

FaxNOW +44 1256 329728
✉ val.kane@oxoid.com

Chocolate has recently been added to the list of products recalled from supermarkets after testing positive for salmonella (Shutterstock).

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