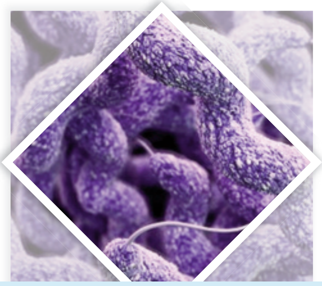


Number 1

FOCUSING
on Campylobacter in poultry

by Dr Roy Betts, Campden BRI. www.campdenbri.co.uk

Campylobacter has always caused large numbers of isolated cases of food poisoning. Over the last few years it has been acknowledged that the organism is now responsible for increasing numbers of outbreaks of illness. Poultry is recognised as a source campylobacter but the risk of human infection from poultry meat can be drastically reduced by production practices, good hygiene and proper cooking. Strategies implemented by industry and retailers to minimise the presence of campylobacter on poultry appear to be showing success.

Campylobacter are Gram negative, narrow, long, rod-shaped bacteria. C. jejuni, C. coli and C. lari appear to cause 90% of reported campylobacter-related human illnesses.

Campylobacter grow best at 37-42°C. They cannot grow below 30°C, so while they can be carried on chilled foods, they are not able to grow on them.

What does it cause?

Those that consume viable campylobacter in foods may suffer from campylobacteriosis. It has been estimated that consuming as few as 500 of the bacteria may be sufficient to cause illness.

The most consistent symptom of campylobacter infection is diarrhoea which may contain blood.

Other symptoms include fever, nausea, vomiting, abdominal pain, headache, and muscle pain. The majority of cases are mild, do not require hospitalisation, and are self-limited. However, Campylobacter jejuni infection can be severe and life-threatening.

Campylobacter and poultry

Campylobacteriosis is a zoonosis – its original source is animals. The bacteria usually live in the intestines as part of the animal's normal flora, and is shed in the faeces. With a few exceptions, campylobacter species do not cause any signs of illness in the animal host.

Humans can become infected through direct animal contact, by drinking water contaminated with animal faecal material, through drinking contaminated milk and by eating contaminated meat products.

Of all meat products, poultry carries the highest risk of carrying campylobacter and is arguably the cause of most cases of campylobacter related food poisoning.

EFSA estimates that poultry and poultry meat account for 20-30% of human cases of campylobacter food poisoning in Europe, whilst the UK FSA estimates that poultry causes 60-80% of cases in the UK.

A recent baseline survey on poultry, carried out by the European Food Safety Authority reported that campylobacter was detected in pooled caecal contents of broilers and on broiler carcasses in all 26 of the participating EU Member States and the two participating non-Member States.

Overall the prevalence of campylobacter-colonised broiler batches was 71% and that of campylobacter-contaminated broiler carcases was 76%. Member State prevalence varied from 2-100% and from 5-100%, for caecal contents and carcases respectively.

A UK study in 2007-2008 and reported by the UK FSA noted a campylobacter prevalence at retail in the UK of 65%.

Reduction strategies

The UK FSA is currently working with the poultry industry and food retailers to reduce levels of campylobacter on raw poultry.

In 2010 the FSA set targets for the reduction of campylobacter in UK retail poultry; the targets were based on three contamination levels:

- <100/g.
- >100 to 1000/g.
- >1000/g.

There was a target to reduce the number of retail poultry samples in the highest category (>1000/g) from its 2008 level (27%) to 10% or lower by the end of 2015.

The FSA modelling suggested that by reducing the numbers in the higher contamination level, the number of human cases of campylobacteriosis linked to poultry should fall by 50%.

In order to monitor this within the UK, the FSA set up an extensive surveillance project to look at contamination levels in raw poultry on retail sale (Table 1).

The variations seen in the whole year figures for 2014/15 and the quarterly figures in 2015/16 are probably due to the well known seasonality of campylobacter contamination, which is known to vary on annual cycles. It is only when the final March to July figures for 2016 are published that we will fully understand the year on year comparison. However there does appear to be a notable reduction in both the total number of retail samples contaminated with campylobacter and those at the highest level of contamination.

The rate of human infections with campylobacter reported in England, Wales and Scotland in 2016 was lower than in previous years, which may indicate the campylobacter reduction programme is having some initial success.

In March 2016 the UK FSA outlined the next stages of its proposed campylobacter reduction plan.

It was believed that the >10% target for the most highly contaminated samples would be reached by the end of 2016, and that by the end of 2017 they wanted to reduce the level of human cases by 100,000/year (i.e. from 280,000-<180,000 cases).

Campylobacter controls

Governments and food producers have been working together to try to reduce the levels of campylobacter in poultry. Poultry producers and food retailers have established better production methods e.g. looking at the effects of thinning in poultry houses.

There have also been a number of research projects considering the surface treatment of carcases.

Techniques such as surface freezing and application of steam and ultrasound have all been considered and had some success. The development of roast-in-bag poultry has also helped as it eliminates the need for the handling of raw poultry within domestic kitchens, preventing cross contamination.

A proportion of poultry is sold precooked. Issues of industrially produced cooked poultry being linked to any cases of food poisoning are almost non-existent due to strict hygiene standards, proper cooking and separation of raw from cooked meat.

In smaller kitchens, it is essential to ensure raw poultry, its packaging material and juices do not come into contact with ready to eat foods (RTE) or the areas and utensils used to prepare or store the RTE foods.

Proper cooking of raw poultry and poultry products (to centre temperatures of at least 70°C for two minutes) will eliminate viable organisms. Cooked poultry must then be stored in a way that prevents recontamination.

Conclusions

Campylobacter cause more outbreaks and cases of foodborne illness than any other organism. The main risk in human foods is contaminated raw poultry.

Over recent years a great deal of work has been done to understand and control campylobacter levels in raw poultry and this is having some positive effects. The correct cooking of raw poultry and poultry products, and then storage to prevent post cooking contamination, is essential, as is good personal hygiene. All of these will considerably reduce the risk of campylobacteriosis. ■

Table 1. Campylobacter results from the FSA surveillance project.

Dates	Samples positive (%)	Samples >1000/g (%)	Outer packaging positive (%)
Feb 14-Feb 15 (Whole year)	73	19	7
July 15-Sept 15 (Quarter year)	76	15	6
Oct 15-Dec 15 (Quarter year)	59	11	5.7
Jan 16-Mar 16 (Quarter year)	50	9.3	5.6

Number 2

FOCUSING
on E. coli in fresh produce

by Dr Roy Betts, Campden BRI. www.campdenbri.co.uk

In recent years Shiga Toxin-Producing E. coli (STEC) have become recognised as a group of very potent human pathogens. Their first association with foods came in the 1990s with cases of what was then a 'new' human pathogen, E. coli O157, linked to meat products, particularly undercooked beef burgers. The illness caused by this organism was severe, with bloody diarrhoea, haemolytic uraemic syndrome leading to kidney failure and, in the worst cases, the death of those affected. It was not long before this 'burger bug' started to be linked with other food products, including fresh fruits and salad vegetables.

One of the first fruit-related issues with the organism occurred in the USA with unpasteurised apple cider (unfermented, unpasteurised juice) in 1991. This was a small outbreak affecting 23 people; however, in 1996, a much larger issue arose with an apple juice from a larger producer. This resulted in 70 people affected, of which 23 were hospitalised and one died.

The issue arose when fallen apples were used to produce the juice; the meadow into which the apples had fallen had been grazed by deer. The apples had become contaminated by faecal material, and the deer faeces contained the E. coli. The problem was clear, the use of apples from the ground increased the risk of faecal contamination, and pathogenic E. coli can be present in soil.

Since those early days the number of fresh products linked to outbreaks of E. coli O157 has grown. Perhaps the first outbreak linked to lettuce was in Montana in 1995 when 70 people were reported ill. The outbreaks have continued to occur virtually on an annual basis ever since, with a wide range of different leafy greens. One of the largest occurred in 2006 and involved spinach from California.

This resulted in over 200 cases and three deaths. The investigation of the outbreak concluded that the fields used to grow the spinach were often crossed by wild pigs, and that these were likely to be the origin of the contamination.

In other parts of the world even larger problems occurred. In 1996 in Sakai, Japan, somewhere in the region of 8,500-10,000 illnesses due to E. coli O157 occurred, linked to eating fresh radish sprouts. The sprouts were served to children in school lunches and so illness affected a vulnerable population that were more susceptible to infection. Since that time sprouted seeds have become another product widely associated with E. coli O157.

Contamination of sprouted seeds often originate from contamination of the seed itself, usually because of growth or harvesting in less than optimal hygienic conditions. Because the seed is dry, contaminating organisms cannot grow. However, during the sprouting process, seeds are irrigated with warm water for some days, ideal conditions for microbial growth, thus considerably increasing risks of illness if human pathogens are present.

Before further discussion of incidents related to sprouted seed, it is necessary to look in more detail at the STEC group. So far only issues involving E. coli O157 have been described as this was the first STEC to be regularly associated with illness. There are, however, many other types of E. coli that fall into the STEC group.

STEC is the name of a group of pathogenic E. coli that contain genes coding for 'shiga toxin' (stx). The presence of this toxin, together with some other pathogenicity factors such as the genes allowing the organism to attach to the gut wall and damage it, are responsible for the main symptoms of illness: kidney damage (stx) and bloody diarrhoea (gut wall damage).

Any E. coli belonging to the STEC group has the potential to cause quite severe human disease. This was shown clearly in an outbreak in Germany in 2011, originating from sprouted Egyptian fenugreek seeds. This featured nearly 4000 illnesses and 53 deaths. It was caused by E. coli O104:H4 a member of the STEC group that had previously caused few cases of human illness.

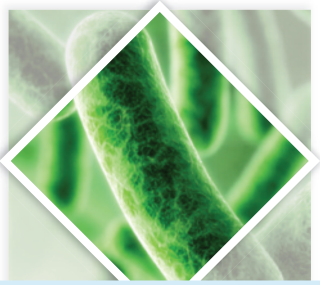
This one outbreak resulted in the European Union demand that all sprouted seeds be tested for STEC before being placed on the market, and there is now a legal requirement to test them for E. coli serogroups: O157, O26, O103, O111, O145 and O104:H4. This has led to microbiology laboratories such as the one at Campden BRI having to set up specialist pathogen handling laboratories and validate complex methods for the detection of STEC from foods and gain ISO17025 accreditation for such methods.

In the UK there have been outbreaks of E. coli O157 linked to watercress in 2013 and mixed salad leaves in 2016. The watercress outbreak affected about 20 people and was thought to have originated when manure contaminated water run off from a nearby cattle farm entered the watercress beds. The 2016 outbreak was responsible for over 160 illnesses, but no root cause was ever confirmed.

In conclusion, since the early 1990s a wide range of fresh produce has been linked to outbreaks of STEC illness. STEC can cause very large outbreaks and the symptoms can be very severe. However, much can be done to reduce the risks of such issues occurring. In a vast majority of the issues discussed, the root cause of the problem has been contamination of the produce with animal faecal material. By controlling contact between animals and fresh produce destined to be consumed raw, the problem can be greatly reduced.

Preventing animals getting into produce growing areas, strict control of irrigation waters and methods of irrigation, good hygiene during harvest and in the handling of harvested material, and the well controlled use of hygienic produce washing will all control the risks of this occurring.

Fresh produce is a highly nutritious part of our diet. It is consumed in large amounts and actually causes very few illnesses; the STEC issues noted here are rare, but can be severe and need to be understood and controlled. By doing this we can assure a safe food supply with a low risk to consumers. ■



Number 3

FOCUSING on Salmonella and pork

by Dr Roy Betts, Campden BRI. www.campdenbri.co.uk

Salmonella is arguably the best known of all food pathogens. The history of salmonella's discovery is interesting and closely associated with pigs. In 1885, D.E. Salmon, an American bacteriologist, isolated an organism that he named Bacillus cholera-suis from pigs suffering from hog cholera. Similar organisms were isolated from outbreaks of foodborne illness, and the Genus Salmonella was named in 1900 in honour of Salmon.

Salmonella is a zoonotic organism. It is derived from animals and is passed by various vectors to humans who consume it in foods or drinks. The types of vector are numerous, and large numbers of different food types have been linked to salmonellosis. Foods that originate from an animal source, such as raw meats and milk, have all been linked to outbreaks of salmonellosis.

Raw fruit and vegetable materials that may have come into contact with animals or animal products (for example untreated manure, other faecal materials, or contaminated irrigation water) may also become contaminated, as can water supplies due to run off from contaminated land.

The food type that has been most closely associated with human outbreaks of salmonellosis is poultry. In recent years, however, contamination levels in poultry and eggs has declined and there have been similar decreases in rates of human illness linked to poultry related strains of salmonella. This has refocused the view of food safety professionals onto other sources of salmonella.

The 2016 European Union report on Zoonoses showed that salmonella is second only to campylobacter as a cause of human food poisoning with 94,000 reported cases in 2015, this being a 1.4% increase over 2014 figures.

As in previous years, the two most commonly reported salmonella serovars in 2014 were Salmonella enteritidis and Salmonella typhimurium, representing 45.7% and 15.8%, respectively, of all reported serovars in confirmed human cases. S. enteritidis is predominantly linked to poultry, whilst S. typhimurium is linked with a range of sources including red meats.

Considering the 2015 European data on salmonella prevalence in meats, the organism was most frequently detected in broiler meat (6.5%) and turkey meat (4.6%), whereas a lower number of positive samples were found in pig meat (1.7%) and bovine meat (0.2%).

Salmonella was rarely found in table eggs (0.9%, in single samples). Ready-to-eat (RTE) foods can pose a direct risk to consumers, and 1.1% and 0.7% positive samples were found for RTE food from meat from broilers and pig meat, respectively.

This data indicates that whilst salmonella levels in poultry are declining rapidly, it is still the primary animal source of salmonella. However pork appears next on the prevalence list, ahead of bovine sources.

Data on the prevalence of salmonella in European herds is available, but reports note the data should be treated with caution as there are no harmonised control programmes in Europe and therefore the data is incomplete. However, the information that is known for red meats indicates the herd prevalence for salmonella is 12.4% for pigs and 2.1% for cattle.

Considering the types of salmonella found in pigs S. typhimurium accounted for 56.9% of isolates reported in 2015, whilst S. derby was the second most common serotype, accounting for 13.7% of isolates. The same serotypes were also identified as the most frequent from pork meat – S. derby accounted for 22.9% of the isolates from pork meat, followed by monophasic strains of S. typhimurium (22.3%) and S. typhimurium (20.6%). This is important information to consider because, as noted previously, S. typhimurium is the serotype that causes the second highest number of human cases of salmonellosis in the UK.

It is clear that numbers of cases associated with S. enteritidis (the greatest cause of human salmonellosis) have been falling gradually year on year, whilst cases associated with S. typhimurium appear to be fairly static.

Antimicrobial resistance

A growing concern within public health bodies is the increasing levels of antimicrobial resistance seen in a variety of isolates of foodborne human pathogens, including salmonella.

For serious cases of bacterial infection the main control used by healthcare specialist is the use of antibiotics. These act selectively on bacteria and will quickly and effectively kill them, allowing those affected to recover well.

Over recent years there has been an increase in the numbers of pathogen isolates showing antibiotic resistance; these organisms cannot be controlled by various antibiotics and pose a real risk to human health.

The EU has been monitoring the levels of antibiotic resistance in order to produce benchmarks against which increases or decreases in bacterial resistance can be measured over time. There is data available on the resistance of salmonella isolates from pigs, the latest being published as part of the EU's 2015 report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food.

Among the Salmonella spp. isolates from pig meat, the highest levels of resistance were noted to the antibiotics ampicillin, sulphamethoxazole and tetracyclines. It was noted that resistance was at 'high to extremely high levels' in most of the Member States included in the analysis (overall, 44.7%, 48.5% and 49.1%, for these three antibiotics respectively).

In Salmonella spp. isolates from bovine meat, resistance to the majority of the antimicrobials tested were lower than those observed in pig meat.

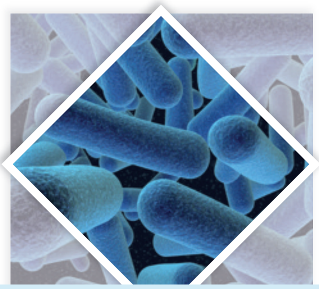
Overall, the situation with salmonella in pigs and pig meat is one to remain aware of. Contamination levels are still below that seen in poultry, but of the red meats pigs and pork would appear to be above those of bovine sources.

Salmonella is, of course, easily controlled, even in contaminated meats, by correct hygienic handling and proper cooking (the centre temperature of the meat achieving at least 70°C for two minutes).

However, the past issues with poultry contamination are a warning. If contamination of any meat is at a high level with a high prevalence, and if it is assumed that a small proportion of meat will be improperly handled (cross contamination occurs in the kitchen) or not thoroughly cooked by consumers, then illnesses will arise.

It will be important in the future to continue to monitor salmonella occurrence in pigs and pork to be able to understand if this is a declining, static or increasing issue.

The proportion of salmonella isolates that show types of antibiotic resistance also needs to be carefully reviewed. In the meantime, those handling and preparing pork and pork products should use good hygienic preparation measures and always ensure they cook the meat properly. In that way, the consumer will be assured of a good quality and safe product. ■



Number 4

FOCUSING on listeria

by Dr Roy Betts, Campden BRI.
www.campdenbri.co.uk

Listeria are common organisms. They have been isolated from a range of different animal species and can be often be isolated from varied environmental sources such as soils and waters. Until 2006, the Genus Listeria contained six species (L. monocytogenes, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri and L. grayi), however through the use of newer DNA based identification systems, at the end of 2016, we were at the point of recognising 17 different Listeria species. Of these, only L. monocytogenes is considered to be a foodborne pathogen.

Before the mid 1980s, listeria were not considered to be a food related problem. Testing of foods was never done, the organism being of interest only to research and veterinary microbiologists. From the mid 1980s, human listeriosis was seen to be increasing and the link to foods, particularly chilled ready-to-eat foods (RTE), was made.

A range of chilled RTE foods were implicated or associated with outbreaks of listeriosis including: soft cheeses, pate, cooked meats, smoked fish, salads, coleslaw, ready made sandwiches and even butter.

The trend in L. monocytogenes related foodborne issues was easy to follow. Listerias do not compete well with other bacteria and in warmer conditions will be outcompeted in most environments.

However they are exceptionally good at surviving and growing in cool, wet conditions and can be very difficult to eradicate once established.

Once the organism inhabits such an environment, it can contaminate foods prepared in these areas and continue to grow even in products stored under very good chilled conditions in retail chillers or domestic fridges.

The role of L. monocytogenes as a hazard in chilled ready-to-eat foods was established, and food safety professionals knew what to look for and where to look. That is when listeria began to become elusive.

In 2011 the USA began suffering one of its largest outbreaks of listeriosis ever recorded with 146 reported illnesses and 30 deaths.

The source of this outbreak had nothing to do with RTE meats, cheeses, pates or fish, but arose from pre-cut cantaloupe melon.

How did melon cause such a large outbreak?

The US Food and Drug Administration (FDA) indicated that it arose through a mixture of low level field contamination, inadequate cooling of the harvested melon, a packhouse design that allowed water to pool and was difficult to clean, and potential cross contamination of melon from other raw agricultural products handled in the packhouse.

The pH of melon will be around 6.0-6.6 so well within the growth range of listeria. Once contaminated, even if stored under chilled conditions, L. monocytogenes would be able to grow and cause a large outbreak from an unexpected source.

As well as the human cost, severe illnesses and deaths, the producing company went bankrupt and the company owners were charged with criminal offences.

Moving to 2015, again in the USA, 35 cases of listeriosis and at least three deaths were linked to caramel (toffee) apples. Technically listeria should not grow in this type of product. Apples are too acidic and caramel has a water activity that is too low for listeria to grow. So what went wrong?

Research done since the outbreak has indicated that if apples are deliberately contaminated with L. monocytogenes and punctured with a stick then coated with caramel, instead of being directly coated with caramel, only those with the stick show significant growth of listeria.

It is believed that puncturing the apple with a stick released moisture from the apple that became trapped between the skin and the caramel coating.

This trapped moisture can then become a micro-environment that allowed the growth of L. monocytogenes to higher levels than is possible in un-punctured apples.

It is clear from this that sometimes our preconceived ideas about where listeria can or cannot grow can sometimes be wrong, and that even in products generally considered safe, we only need small changes to the conditions within the product, to produce an environment that can allow listeria to grow.

Have there been other issues? In 2016 in the USA there was a recall of sunflower seeds due to contamination by L. monocytogenes.

Again the water activity of these seeds would be too low to allow growth to occur, but simply the presence of L. monocytogenes in such a RTE product within the USA is enough to generate recall notices.

The issue with a product like sunflower seeds is that they are used as an ingredient in many different products.

So the original recall of seeds triggered a cascade of recalls of many other product types, such as: trail mix, nut bars, sunflower seed butter, granola bars and various types of snack mixes.

Listeria in frozen produce

We also know that listeria will not grow in frozen products as the temperature is too low. That did not prevent a large recall of frozen fruits and vegetables in the USA in 2016 that were thought to be contaminated with L. monocytogenes.

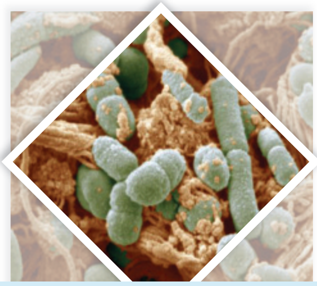
The issue here was that freezing will preserve bacteria and there was the possibility that consumers would use frozen vegetables such as peas, beans and sweetcorn in salads, without cooking them. It is believed that such contaminated frozen foods caused an outbreak affecting nine people.

In conclusion, it is clear that food safety professionals need to expand their views on the types of food liable to cause L. monocytogenes related problems.

Chilled ready-to-eat foods will remain an issue, but fruits, dried foods, frozen materials and perhaps other food types must now also be reviewed with respect to L. monocytogenes. ■

Number 5

FOCUSING on antimicrobial resistance



by Dr Roy Betts, Campden BRI.
www.campdenbri.co.uk

Bacteria are everywhere in our world, on our skin, in our foods and on every surface we touch. Chemical agents that kill bacteria have been well known for many years and have saved many lives. However, many of these are not selective and will kill any living cell they contact, including human cells. The ideal is a chemical agent that eliminates bacteria but not other cell types.

In the early 1900s, Paul Ehrlich worked on a series of dyes that stained certain bacteria but not other cells. He developed the idea of selective killing and through a highly structured research programme produced the first really effective treatment for syphilis.

It was not until 1928 that the first true antibiotic – penicillin – was discovered by Fleming after noting how a culture of the mould Penicillium could inhibit bacterial growth.

By 1945 penicillin was being mass produced to treat those affected with bacterial infections.

Many types of antibiotic were discovered between the 1940s to the 1970s but concerns were already being raised about the potential for bacteria to become resistant to the agents.

Resistance occurs primarily because of the genetic diversity of bacterial populations and their fast rate of growth. In a large population of bacteria, a very low number may be resistant to an antibiotic.

When treated with that antibiotic, the sensitive bacteria will be killed but the rest will survive and be able to quickly grow (vertical transmission of resistance).

Some groups of bacteria can also pass their genetic resistance directly onto other organisms (horizontal transmission of resistance).

Link with foods

But how does antimicrobial resistance (AMR) link to foods?

In 2016, the UK Food Standards Agency published an extensive review of the issue. Animals destined for the human food chain are exposed to antimicrobials in a number of ways: they can be used to prevent infections; as treatments for infections; and in some countries (although not in the EU) as growth promoters.

Any use of antibiotics has the potential to create a large AMR population of bacteria. The only way to limit AMR is by controlling the use of antibiotics.

Antimicrobial use in animals is strictly controlled in the EU, but outside of the EU this is sometimes not the case. This could potentially lead to animal based foods being contaminated with resistant bacteria, and entering the human food chain.

Foodborne pathogens with AMR, such as fluoroquinolone-resistant Campylobacter spp. and extended-spectrum β-lactamase (ESBLs)-producing bacteria have been isolated with increasing frequency in food, food-producing animals and humans in Europe.

An EFSA review in 2015 noted that 54% of isolates of C. jejuni from both broilers and humans were resistant to ciprofloxacin (an important fluoroquinolone antibiotic), whilst that level rose to over 66% for resistance in C. coli.

Considering that campylobacter is still the largest cause of food poisoning in a majority of EU countries, this is a major concern in any cases where antibiotic therapy may be considered.

Some bacteria are resistant to multiple antibiotic types. These organisms are known as multi drug resistant (MDR) strains and are of particular concern.

There has been a rise in the number of MDR salmonella. EFSA recently reported that MDR salmonella frequently detected from humans and animals (and from meats derived from those animals).

MDR salmonella strains were found in 32% of human isolates, 56% of broilers, 73% of turkeys and 38% of pigs. This is of concern as MDR strains can be particularly difficult to control if found to be causing problematic infection.

Until recently, resistance to colistin antibiotics was believed to be transmitted only vertically (between the same type or strain as they divide), but there is now evidence for horizontal transmission across bacterial strains via plasmid-borne resistance.

Plasmids are small pieces of DNA that are contained within some bacteria. They are not part of the organism's chromosome. It is possible for plasmids to divide and to move from one cell, to another nearby cell spreading AMR to other organism types. Colistin-resistant Escherichia coli with plasmid-mediated resistance has been recently reported in Denmark in imported frozen poultry and transmission through the food chain to humans of such organisms has been demonstrated.

Plasmid-mediated resistance to colistin has also been identified in salmonellosis cases in humans in other European countries. This resistance trait has also recently been observed in pig farms in the UK and in E. coli isolates from broilers and turkeys in several European countries.

Horizontal transmission

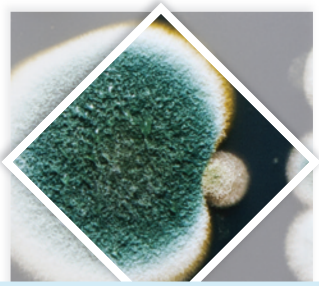
The potential for horizontal transfer of AMR genes within the population of the gut has been the focus of recent research. The human gut contains a wealth of bacteria, many will be unculturable and therefore unknown to us. Developments in DNA based testing have resulted in the technique of metagenomic analysis which allows a study of population without a need to culture.

This and other DNA based techniques are revolutionising our knowledge of complex populations such as those in the gut. We now know that the normal gut flora contains an array of different antimicrobial resistance which has been named the 'resistome'.

Our concerns centre on horizontal transfer of such AMR elements between organisms/strains. While the presence of AMR in a harmless commensal organism is of no consequence, if that resistance is transferred to a pathogen, it is of great significance and concern.

The development of AMR in pathogens is of great consequence. Disease severity and mortality will increase if we cannot control infections using antibiotics.

We need to keep surveying the types and spread of AMR throughout animals, foods and the human population, and as far as possible limit antibiotic use to prevent the development and spread of resistance. ■



Number 6

FOCUSING

on yeasts & moulds

by Dr Roy Betts, Campden BRI. www.campdenbri.co.uk

Yeasts and moulds form an important group of organisms of great importance to the food industry. These groups are very different to the bacteria that are commonly associated with food, being eukaryotic organisms similar to cells found in plants and animals. The yeasts and moulds are widely dispersed, being found in a variety of locations and are virtually ubiquitous in any environment.

Whilst the yeasts and moulds form a single similar group of eukaryotic organisms, they are in fact quite different in form. The yeasts form a group of single celled organisms, that reproduce by a process known as budding, where 'daughter' cells bud off the parent cell forming new single cells.

Yeasts tend to grow within food and drink matrices in planktonic form and they tend to ferment sugars growing well under anaerobic conditions.

Moulds exist in a multicellular format usually known as a hyphal mat, they tend to grow on the surface of food and drinks and not within them as yeasts do. They are aerobic organisms and cannot grow well under conditions in which oxygen is limited.

Yeasts

Yeasts are very important within food microbiology as they can create both positive and negative effects. Where would we be without bread, beer and wine, all created by the fermentative action of yeasts?

Conversely the ability of some yeasts to survive and grow at low pH, low water activity, and in the presence of some common chemical preservatives, make them potent food spoilage organisms responsible for large economic losses of some food products.

The origin of the positive effects of yeasts in food production, probably came about by an accidental contamination of some raw materials with environmental yeasts. Mead, a fermented honey drink, is the oldest alcoholic beverage known to man and is believed to have been discovered during the Stone Age.

A chance occurrence of honeycomb becoming wet from rain and then airborne yeasts fermenting the mixture is thought to have led to its discovery.

Leavened bread first appeared in Egypt about 5,000 years ago, when flat bread dough became contaminated with wild yeasts which would have produced carbon dioxide, and 'raised' the bread.

No doubt an 'accidental' contamination of various fruit juices would have caused the production of wines.

Today, contamination of raw materials with wild environmental yeasts is still used to produce some foods such as specialist sourdough breads and lambic beers.

However, most food production that uses yeasts will now utilise specialist strains obtained from culture collections, that are cultured and deliberately inoculated into their growth substrate to create the food required

Today in food production, yeasts are more usually linked with food spoilage.

Yeasts are slow growing organisms when compared to bacteria. If yeasts and bacteria were placed in the same optimum environment and both could grow, it is most likely that the faster growing bacteria would quickly outgrow and outcompete the slower growing yeast, becoming the dominant flora.

However, if we move outside the 'optimum' growth conditions of most bacteria, into environments that are acidic, or of low water activity (for example high in sugar), then the yeasts have the advantage and would rapidly overtake the growth of bacteria.

It is in these specialist niches in foods, that the spoilage yeasts become a problem.

Yeast growth

Yeasts are generally associated with the fermentation of sugars such as glucose and sucrose, but they are able to utilise a variety of other compounds, such as alcohols organic acids, hydrocarbons and aromatic compounds.

Some yeasts are also capable of utilising certain acid-based preservatives such as benzoic acid, propionic acid and sorbic acid, and this can make them a major issue in foods and drinks that rely on these preservatives for stability.

Other general environmental factors that influence growth are temperature and the concentration of oxygen.

The temperature range for yeast growth is about 0-47°C, with yeasts from Antarctic soils for example, having a maximum growth temperature of 17°C, whilst some from tropical environments will grow at greater than 40°C.

Some yeast species are strict aerobes whilst others also have a fermentative metabolism.

In the case of a contaminated fruit juice for example, fermentative yeasts will cause alcoholic fermentation in the bulk of the product, whilst aerobic yeasts will produce a film or pellicle on the surface of the liquid.

In most food spoilage it is the anaerobic fermentative yeasts that cause the major issue. In sealed food containers, any oxygen will be rapidly consumed creating an anaerobic environment, and it is here that the spoilage organisms will grow, the most characteristic spoilage event being gas production.

Flexible containers will become distended, whilst rigid containers appear unaffected until opened when rapid pressure release can result in a forcible ejection of the contents.

The osmophilic or xerotolerant yeasts specialise in growing in environments of high osmotic pressure, due to the presence of salt or sugar.

Many of the yeasts isolated from high salt environments will grow readily under salt-free conditions, but this is not the case for those organisms isolated from high sugar environments, which grow poorly or not at all on standard growth agars.

Syrups, jams, conserves, fondants are all susceptible to spoilage by xerotolerant yeasts.

Moulds

Like the yeasts, moulds can also produce both positive and negative effects in foods.

Their negative effects are well known, mould contamination of products containing high sugar or of low pH is obvious with the organisms tending to grow as colonies on the surface of such products. Their positive effects are also well known.

Moulds will be seen on the surface of mould ripened cheeses such as brie and camembert, and within blue veined ones like stilton and Danish Blue.

Some species are a key part of some fermented food products from Japan, whilst the meat replacement known as Quorn, is produced from a Fusarium mould that is cultured and treated to form a 'meat like' texture.

On the negative side, mould like yeasts can affect low pH and low water activity foods, indeed this group can grow at very low water activities (Aw) causing spoilage problems in products with Aw values below 0.75.

Mould growth

Moulds are slow growing organisms and will be rapidly out competed by bacteria and even yeasts in normal conditions. They come into their own when the pH and/or Aw is reduced and other groups are unable to grow.

Then the moulds can take over, forming hyphal mats or colonies on the surface of food products. It is important to realise that moulds are aerobic organisms, they need oxygen to grow, so they will usually be confined to the surfaces of foods, forming easily visible, often coloured colonies.

Airborne yeasts

Yeasts which are present in the air must be capable of surviving very harsh conditions.

Airborne yeasts can be present in the form of ascospores and although these are more resistant than vegetative yeast cells, the majority of yeast particles present in the air will not be viable.

Soil, dust, drains, equipment surfaces, raw materials and ventilation ducts can all release yeasts into the air. Viable counts from settle plates reflect a dynamic situation of yeast particles becoming airborne and then settling again.

The size of the yeast particle determines how far it will travel before settling, with most airborne particles being between 2-20μm in size.

As a source of product contamination, airborne yeasts are of most concern for such operations as aseptic filling plants and in these cases control of the air quality by means of air filtration will be necessary.

Contamination of plant and processing equipment

A lack of attention to the hygienic design of factory plant and cleaning and sanitation procedures can lead to yeast and mould contamination of products.

This is particularly problematic in plants producing high sugar/low water activity/low pH products. Factories producing fruit products, baked goods, confectionery, fermented dairy products etc can be at real risk from yeast and mould contamination.

In such plants care must be taken to ensure that equipment such as proportioning pumps, hose connections and valves, storage chillers, and other product contact surfaces are adequately sanitised.

Factories should be designed in such a way as to reduce the risk of cross contamination of the finished product by the raw product.

In many cases it is assumed that low pH products are quite stable due to an inability of bacteria and many yeasts to grow in such conditions. However it only takes very low levels of specialist spoilage yeasts and moulds to create major spoilage problems in these products.

Such organisms are ideally suited to the environmental niches in these production environments, hence the need for good hygiene in such areas.

Contamination of packaging materials

It is of considerable importance to consider the hygienic status not only of the product and production equipment, but also of any containers and packing materials, as these too can be a source of spoilage yeast and moulds.

Any purchased containers, for example jars and bottles and container closures (lids/caps), have to be assessed for their level of contamination and any need for decontamination before use.

The storage of such containers/closures before use is also important. On a number of occasions the storage of opened pallets of containers in open/semi-open environments drastically increases the potential for contamination.

Cardboard may be highly contaminated with environmental yeast and mould spores and even foils and plastics may show low levels of contamination.

Yeast numbers may increase substantially during storage, unrolling and moulding due to static electricity attracting dust from the environment.

Resistance and human illness

Moulds and some forms of yeast are able to produce spores, and these can be heat resistant. This make the determination of any heat process used on food products very important.

It is not unknown for heat processed low pH/low Aw products to be spoiled by moulds whose spores have survived heat processes able to destroy vegetative bacteria. Generally yeast and mould spores are not as resistant as bacterial spores, but are much more resistant than vegetative bacterial or yeast cells.

Companies wishing to establish heat processes able to destroy such organisms are advised to contact specialist advisors who can help to establish suitable heating processes.

Whilst yeasts are not usually acknowledged to cause foodborne illnesses, some moulds can produce a range of mycotoxins that can have chronic effects.

Unacceptable levels of many mycotoxins are detailed in legislation, and this should be consulted if moulds are considered a potential problem in particular foods types

Testing methods

It is of great importance to ensure raw material, the production environment and the final product are subject to testing for potential spoilage yeasts and moulds. This, however, is not as easy as it sounds.

Whilst 'normal' yeasts and moulds may be easily grown on media such as Rose Bengal Chloramphenicol Agar (RBCA) or Malt Extract Agar (MEA), specialist spoilage organisms present in low water activity or low pH environments may not.

It is usual for low water activity foods to be tested using media such as DG18, and even the initial dilution of the foods may need to be done using a specialist diluent, containing levels of sugar.

This will prevent the yeasts and moulds being injured by osmotic stress. Injured cells may not grow, will not be detected and thus a possible problem will remain hidden, until product begins to spoil.

It is recommended that when setting up testing regimes for such organisms, expert help is consulted on methods required. ■