SOFHT Breakfast Club

The Importance of Pathogenic Bacteria

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Pathogenic Bacteria

• Pathogenic bacteria are defined as those capable of causing illness by any means – including medical bacteria and food poisoning organisms.

• This talk is only concerned with food poisoning organisms and food-borne illness.

• Importance of pathogens is two-fold
  – Effects on the sufferer
  – Effects on the food producer
Effects of foodborne disease on people

- Illness, distress of the patient, effects on the family, possible loss of earnings, possible cost of treatment.

- Loss of working days, cost to the employer of overtime or agency staff to cover absence.

- “Figures released in the Daily Mail revealed that 22 million working days a year are lost to food poisoning, while treating related illness costs the NHS and business £750 million / year”.

- Who is at risk? We are all exposed to food-borne pathogens. Anyone can become ill, not everyone does. The young, the elderly and those with weakened immunity are most likely to be affected.
Effects on the Food Producer

- Implications of illness linked to a company or brand name. The food producer will suffer financially and could lose their reputation and major contracts.

- Product recall can be very costly and very public.

- Sources of problems. A pathogen may come into a food production unit with ingredients or pests, and may colonise the equipment or infect the staff. Carriers risk contaminating the product particularly if personal hygiene practices are poor.
Food Poisoning Bacteria

- Salmonella
- Campylobacter
- E. coli O157
- Staph aureus
- Clostridium perfringens
- Bacillus cereus
- Vibrio parahaemolyticus
- Foodborne illness – Listeria - not poisoning
Major Food-borne Pathogens

Campylobacter
Salmonella
E. coli O157

Bacteria causing infections of the digestive system, sometimes from a low dose. Symptoms may include diarrhoea, vomiting, nausea, dehydration, fever.

- **Listeria** is food-borne, but not strictly a food poisoning organism. It presents with a variety of vague symptoms, and a long incubation period. Severe infection with L.monocytogenes can lead to miscarriage, infant meningitis and death in the elderly. 30% mortality.
Toxin Producing Pathogens

- **Staph aureus / coagulase positive Staphylococci**
- **Bacillus cereus**
- **Clostridium perfringens**

These organisms cause illness by producing toxin within the food. They need to have contaminated the food in high numbers to produce enough toxin to cause illness. The toxin is released into the food and may persist after the death of the bacteria and may be heat resistant.
# ACCEPTABLE LEVELS for toxin producers

HPA Guidelines for Ready to Eat Foods

<table>
<thead>
<tr>
<th></th>
<th>Acceptable</th>
<th>Unsatisfactory</th>
<th>Unacceptable, potentially hazardous</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staph aureus</strong></td>
<td>$&lt;100 \text{ /gm}$</td>
<td>$100 – 10^4 \text{ /gm}$</td>
<td>$&gt;10^4 \text{ /gm}$</td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>$&lt;10^3 \text{ /gm}$</td>
<td>$10^4 – 10^5 \text{ /gm}$</td>
<td>$&gt;10^5 \text{ /gm}$</td>
</tr>
<tr>
<td><strong>Clostridium perfringens</strong></td>
<td>$&lt;100 \text{ /gm}$</td>
<td>$100 – 10^4 \text{ /gm}$</td>
<td>$&gt;10^4 \text{ /gm}$</td>
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Food poisoning incidence

- A recent report has quoted a figure of 97,000 cases of food poisoning reported in 2007

- This could be an underestimate by x30

- The Food Standards Agency set a target to reduce food-borne disease by 20% by 2006. Some reduction has been achieved in Salmonella figures. New targets have been set.
Cases with a confirmed cause

1. Diagnosis
2. Sample tested
3. Go to doctor
4. Illness cases
Reported cases of food poisoning 2007

- Total reported cases 72,382
- Campylobacter 51,975
- Salmonella 11,779
- E. coli O157 828
- Listeria monocytogenes 227
- Norovirus unofficial estimate 600,000+ cases

Data from Health Protections Agency for England and Wales
Norovirus

- The size bar is 50nm
- 1nm is one millionth of a millimetre
Improvement in Salmonella figures 1996 - 2008

• The incidence of Salmonella cases reported has dropped significantly in recent years, largely due to the introduction of vaccination of chicks and subsequent drop in S. enteritidis cases.

• Campylobacter levels – stable around 50,000 pa

• E. coli O157 levels – stable around 800-1000 pa
Salmonella incidence 1990 to 2008 (HPA data)
Electron micrograph of Salmonella enteritidis
Regulations and Guidelines

• Food Safety Act – allows prosecution under several clauses.

• Microbiological Criteria Regulations – specify what pathogens must be absent from which foods.

• HPA ready-to-eat Guidelines – no legal status, but used as contributory evidence.

• Industry codes of practise – guidance only.
Microbiological Criteria Regulations

• These are legally enforceable regulations

• Specify absence of Salmonella from meats, dairy products, ready to eat foods, infant formula, shellfish + other items.

• Specify Listeria monocytogenes must not exceed 100cfu/g in ready to eat foods at any point during shelf life, and must be absent from 25gm of infant formula and some other products.

• Exact requirements depend on whether or not the food is capable of sustaining the growth of Listeria. Exceptions exist based on pH and Aw of the food, shelf life and type of product.
Laboratory Aspect of Food-borne Pathogens

- **What is a perfect test method?**
- Accurate & reliable
- Robust, not affected by food type
- Easy to use
- Rapid
- Approved (AFNOR, AOAC); accepted by retailers
- Can accommodate a high throughput of samples
- Able to be accredited as a lab method by UKAS
Types of Method Available for Detection Tests

- Selective enrichment and selective plating
  - ISO methods of culture
  - Culture of target organisms by other means

- Detection by assay – ELISA, VIDAS etc

- Immuno-Magnetic Separation

- PCR

- Rapid identification devices
Traditional Culture Methods

• The challenge is to find a few of the target organism within the whole population of organisms in the food

• Take a representative 25gm sample of the food

• Add a selective broth or a recovery broth to give a 1/10 suspension. Homogenise as necessary.

• Incubate to encourage growth of target organism and inhibition of non-target growth.

• Plate out onto selective / differential agar media, incubate, look for typical colonies.
Traditional Culture Methods

• Depend on being able to successfully grow the target organism and inhibit other types of bacteria.

• Relies on the correct performance of the culture media used and other test conditions.

• Relies on ability of staff to recognise the target organism.

• Suspect colonies undergo confirmation tests

• Both ISO and non-ISO methods follow this general pattern, though the details of media and confirmatory tests may be different.
SUB CULTURE FOR SALMONELLA
**Xylose-Lysine-Desoxycholate Agar (XLD)**

**Typical formula gram/litre**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gram/litre</th>
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<tbody>
<tr>
<td>Yeast Extract</td>
<td>3.0</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>5.0</td>
</tr>
<tr>
<td>Xylose</td>
<td>3.75</td>
</tr>
<tr>
<td>Lactose</td>
<td>7.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.5</td>
</tr>
<tr>
<td>Sodium desoxycholate</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>6.8</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.08</td>
</tr>
<tr>
<td>Agar</td>
<td>12.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient/growth promoting factors</th>
<th>Energy source (carbohydrate)</th>
<th>Mineral salts and metals</th>
<th>Buffer salts</th>
<th>Indicator / dye</th>
<th>Gelling agent</th>
<th>Selective agent</th>
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</table>
Salmonella on XLD

- Well isolated colonies, typical appearance black centre and pink edge.
- Yellow colony to be ignored.
Campylobacter jejuni
Campylobacter culture
Screening Methods

- Enzyme Linked Immuno-Sorbent Assay (ELISA)
- VIDAS

- Depends on enrichment culture as with the traditional culture methods, then detects presence or absence of the specific bacterial antigen in the culture. Detection takes a few hours opposed to 1 to 2 days culture on agar.

- Works by specific antibody reaction leading to a colour change and raised optical density reading.
Screening Methods

- These methods can give a more rapid negative result than routine culture, though if positive would need plating out.

- A negative result is reported as the final result, allowing food to be released.

- A raised OD result is a presumptive positive and the broth must be plated out onto agar. It is not necessarily positive, but needs further testing to obtain the correct result.

- Once the agar culture has grown, confirmatory tests are needed. These may give a positive or negative test result.
ELISA

- Enzyme Linked Immuno Sorbent Assay
- Automated detection method using up to date technology to detect pathogens by use of monoclonal antibodies.
Immuno-Magnetic Separation

- Dynal Dynabead system or Pathatrix system

- Requires enrichment culture in broth to allow bacteria to multiply.

- Broth culture is exposed to magnetic beads coated in antibodies specific to the target organism

- Target organism sticks to the beads, they are harvested using a magnet, rinsed and cultured
Immuno-Magnetic Separation

• Very good for detecting presence of the target organism amongst other bacteria, eg E. coli O157 in raw mince may be present in low levels and greatly out-numbered by other species and other strains of E. coli.

• Antiserum on beads is very specific and captures the target organism. Beads are transferred onto selective agar allowing organism to grow. System removes other bacteria that would mask growth.
Pathatrix equipment
E. Coli O157 on Sorbitol MacConkey Agar
PCR Polymerase Chain Reaction

- High tech detection of organism by analysis of DNA.

- Advantages - highly sensitive, very accurate

- Disadvantages – handling skill required, sample throughput may be slow. Cost.

- Appears to be useful for specific investigations, but application in routine high volume testing is not yet proved to be of benefit. Potential for future expansion.
Rapid Identification Devices

• Market growth area – aiming to give a simple, fast accurate result in kit form. These do not replace the whole of the test but can be a useful screening tool.

• Rapid flow devices require culture in broth, then transfer of a small inoculum onto the device. The culture flows through the device and contacts specific antibodies which react and create a coloured line if positive.

• A second control line shows the test has worked.

• Examples – “Reveal”, “Rapidchek”,

ALcontrol Laboratories
“Reveal” test for E. coli O157
Direct Plating onto Selective Agars

• For pathogens where enumeration is important, suspensions of the food sample and dilutions are plated directly onto the appropriate selective / differential agar. This may be in traditional petri dishes or on dry rehydratable film techniques such as “Petrifilm”.

• Each agar is designed to inhibit unwanted organisms, and encourage the target organism to grow and to have a typical colony appearance.

• Listeria monocytogenes must be <100cfu/gm in ready-to-eat foods at any point in the shelf life. (Regulation).
Staph aureus
Bacillus cereus on PEMBA
Clostridium perfringens
Oxford Listeria Agar

Listeria spp.

Photo courtesy of Oxoid Ltd
Oxoid Chromogenic Listeria Medium (OCLA)

Listeria spp (blue) L. monocytogenes (blue + halo)

Photo courtesy of Oxoid Ltd
Rationale of Method Selection

- For compliance with Microbiological Criteria Regulations*, ISO methods are listed as the “gold standard” methods. Other culture methods are acceptable so long as they have been properly validated against the ISO methods.

- Advantages of ISO methods – accepted method, will be required in the event of a court case.

- Disadvantage, some take longer, may cost more.

- *EC 2073/05, EC 852/04, EC178/02, EC1441/07
International Standards Organisation (ISO) Methods

• Salmonella EN/ISO 6579 : 2002
• Listeria EN/ISO 11290-1: 1997 presence/absence
• Listeria EN/ISO 11290-2 : 1998 count
• Campylobacter ISO 10272 : 2006
• E. coli O157 ISO 16654 : 2001
Rationale of Method Selection

• Routine culture by non-ISO method and screening methods

• Advantage – may be simpler, quicker, cheaper, give an earlier result. Where few positive results are expected, a screening test is useful as negatives can be reported sooner.

• Disadvantage – validation is needed, and the method may not be accepted in cases of legal dispute.

• Customer approval – some major retailers specify which test methods can be used on their products, and will audit the testing laboratories and food producers for compliance.
Rationale of Method Selection

• IMS is the preferred method for E. coli O157. It gives the best chance of recovery of low levels of the target organism from mixed cultures.

• Can be adapted for other organisms, with use of the correct antiserum coated beads.

• Advantage – accuracy, recovery of low levels

• Disadvantage – handling skills, sample throughput
Summary

- Food-borne pathogens are a persistent problem both for the consumer and the food manufacturer.

- Selection of testing methods is influenced by:
  - the likelihood of legal action, need for due diligence
  - the expected quality of the foods,
  - the need to comply with requirements of the retailers,
  - the need for a rapid result,
  - the overall cost of testing.
Any Questions?

- Summer pudding, Dionysius Tavern, Nissi Beach, Cyprus (excellent)