Indicator Organisms

SCI5508
Indicator Organisms

- “REFLECTS” microbiological quality
- “…organisms and/or their metabolic products whose presence in given foods at certain levels may be used to assess existing quality or better, to predict product shelf life”
Need for Microbiological Criteria

- Evidence of Hazard
- Natural microflora
- Processing
- Contamination
- Consumer
- State of Distribution

- Abuse
- Spoilage Potential
- Ultimate Consumption
- Reliability of Detection
- Costs and Benefits
Definitions

• Standards
  – *Listeria monocytogenes*
  – *E. coli* O157:H7

• Guideline
  – *Salmonella* testing
  – Generic *E. coli* Testing

• Specifications
Types of Microbial Indicators

- Aerobic Plate Counts (APC)
- Coliforms
  - *E. coli*
  - *Enterococcus*
- Yeast and Mould Counts
- Can Indicate Quality and/or Safety
Indicators of Quality

• Shelf-life often determined by initial number of organisms present
• NOT a direct correlation
• Must consider
  – Food Type
  – Processes
Criteria - Indication of Quality

• Present and Detectable
• Correlation to product quality
• Detected and distinguishable
• Enumerable in short period
• Not affected by food flora
Criteria - Indication of Quality

♦ Present and Detectable
♦ Correlation to product quality
♦ Detected and distinguishable
♦ Enumerable in short period
♦ Not affected by food flora
APC as an Indicator

- Only measure Live Cells
- Little Value in Organoleptic Value
- Quality Loss May Occur at Low Counts

- MUST know expected values at particular point in process of particular food
APC as an Indicator

- Compliance with Standards or Guidelines
- Purchase Specifications
- Monitor Adherence to GMP or SSOPs
- Monitors various organisms
  - Thermodurics
  - Mesophiles
  - Psychrotrophs
  - Thermophiles
  - Proteolytic
  - lipolytic
Criteria - Indicators of (Food & Pharmaceutical) Safety

- Easily and Rapidly Detectable
- Distinguishable
- Association to Pathogen
- Present when Pathogen Present
- Numbers, Growth and Death Correlate to Pathogen
- Absent from Foods that are Free of the Pathogen
Ideal Relationship Between a Pathogen and an Indicator Organism
Safety Indicators

• Reduce or Eliminate Food-borne hazard
• Applied to Food Products frequently associated with contamination

• Considerations
  – Type of Food
  – Numbers
  – Consumer
Coliforms

• GRAM NEGATIVE, NONSPORE-FORMING RODS THAT FERMENT LACTOSE TO ACID AND GAS WITHIN 48 HOURS AT 37 C
  – KNOW THIS!!!!!!!!!!!
Coliforms

• Family – Enterobacteriaceae
• Genera
  – *Citrobacter*
  – *Enterobacter*
  – *Escherichia*
  – *Klebsiella*
Coliforms

• Growth
  – Temperature
  – pH
  – Nutrients
• Survival
• Distribution
  – E. coli
  – Enterobacter
Finding Coliforms

- Faecal contamination
- Lack of Sanitation
- Increased Likelihood of Pathogen Presence
Limitations of Coliforms as Indicators

- Raw Foods
  - Heat Processed vs Raw Consumption
- Heat Processed
  - Human
Coiform Criteria and Standards

• Large numbers are undesirable
• Difficult to eliminate

• What’s acceptable?
Indicators of Safety - Faecal

- Specificity
- High Numbers in Faeces
- High Resistance
- Easy and reliable Detection
- Non-pathogenic
Faecal Coliforms and E. coli

• Easily destroyed by heat
• May die during Freezing and Storage
• E. coli indicates Faecal contamination and possibly the presence of enteric pathogens
• Failure to detect does not mean pathogen free
Faecal Coliforms

- Production of acid and gas in EC (E. coli) broth between 44 and 46 C
- Fecal Coliforms are E. coli Biotype I
  - EHEC
Enterococci

- Faecal Material
- Plants
- Salt-Tolerant
- Resistant to Freezing
- Few reasons to specify presence of Enterococci within coliform group
Recommendations

• Acceptable and unacceptable ranges of coliform and E. coli counts
• NOT necessarily a regulation, just a guideline
Special Considerations

- **Vegetables**
  - Enterobacter a natural inhabitant
  - Must look at E. coli

- **Meats**
  - Aeromonas and psychrotrophic enterics are coliforms
  - Must look at E. coli

- **Cooked Products**
  - No Coliforms should be present
  - Pasteurized milk
Enterobacteriaceae

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Enterobacteriaceae

• are a large family of bacteria, including many of the more familiar pathogens, – E.g. *Salmonella* and *Escherichia coli*.

• Genetic studies place them among the Proteobacteria, and they are given their own order (Enterobacterales),

• This is sometimes taken to include some related environmental samples.
The Enterobacteriaceae

- rod-shaped,
- typically 1-5 µm in length.
- Gram-negative stains,
- facultative anaerobes,
- fermenting sugars to produce lactic acid and various other end products
The Enterobacteriaceae

- Alishewanella
- Alterococcus
- Aquamonas
- Aranicola
- Arsenophonus
- Azotivirga
- Blohmannia
- Brenneria
- Buchnera
- Budvicia
- Buttiauxella
- Cedecea
- *Citrobacter*
- Dickeya
- Edwardsiella
- *Enterobacter*
  - *Erwinia*, e.g. *Erwinia amylovora*
- *Escherichia*, e.g. *Escherichia coli*
  - Ewingella
  - Grimontella
  - Hafnia
- *Klebsiella*, e.g. *Klebsiella pneumoniae*
  - Kluvyvera
  - Leclercia
- Leminorella
- Moellerella
- Morganella
- Obesumbacterium
- Pantoea
- Pectobacterium
- Candidatus Phlomobacter
- *Photorhabdus*, e.g. *Photorhabdus luminescens*
- Plesiomonas, e.g. *Plesiomonas shigelloides*
- Pragia
- *Proteus*, e.g. *Proteus vulgaris*
- Providencia
- Rahnella
- Raoultella
- *Salmonella*
- Samsonia
- *Serratia*, e.g. *Serratia marcescens*
- *Shigella*
- Sodalis
- Tatumella
- Trabulsiella
- Wigglesworthia
- Xenorhabdus
- *Yersinia*, e.g. *Yersinia pestis*
- Yokenella
Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus

the coliform bacilli
# Taxonomy of Selected Coliform Bacilli and Proteus in Human Clinical Specimens

<table>
<thead>
<tr>
<th>Organism</th>
<th>Other and Older Designations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia</em> (6 species in Bergeys Manual)&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> inactive</td>
<td></td>
</tr>
<tr>
<td><em>E. fergusonii</em></td>
<td></td>
</tr>
<tr>
<td><em>E. hermannii</em></td>
<td></td>
</tr>
<tr>
<td><em>E. vulneris</em></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella</em> (7 species)</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Friedlander's bacillus</td>
</tr>
<tr>
<td><em>K. oxytoca</em></td>
<td>Indole-positive <em>K. pneumoniae</em></td>
</tr>
<tr>
<td><em>K. ozaenae</em></td>
<td><em>K. pneumoniae</em> subsp. ozaena</td>
</tr>
<tr>
<td><em>K. rhinoscleromatis</em></td>
<td><em>K. pneumoniae</em> subsp. rhinoscleromatis</td>
</tr>
<tr>
<td><em>K. ornithopelitic</em></td>
<td>Ornithine-positive <em>K. oxytoca</em></td>
</tr>
<tr>
<td><em>K. planticola</em></td>
<td></td>
</tr>
<tr>
<td><em>Enterobacter</em> (13 species)</td>
<td></td>
</tr>
<tr>
<td><em>E. aerogenes</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pantoea agglomerans. <em>Envelop herbicola</em></td>
</tr>
<tr>
<td><em>E. cloacae</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>E. agglomerans</em></td>
<td></td>
</tr>
<tr>
<td><em>E. sakazakii</em></td>
<td><em>E. cloacae (yellow pigmented)</em></td>
</tr>
<tr>
<td><em>E. gergoviae</em></td>
<td></td>
</tr>
<tr>
<td><em>Serratia</em> (11 species)</td>
<td></td>
</tr>
<tr>
<td><em>S. marcescens</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>S. rubidaea</em></td>
<td></td>
</tr>
<tr>
<td><em>S. liquefaciens</em> group</td>
<td><em>S. liquefaciens, S. grimesii, S. proteamaculans</em></td>
</tr>
<tr>
<td><em>S. ficaria</em></td>
<td></td>
</tr>
<tr>
<td><em>S. lonticola</em></td>
<td></td>
</tr>
<tr>
<td><em>S. odorifera</em></td>
<td></td>
</tr>
<tr>
<td><em>S. plymuthica</em></td>
<td></td>
</tr>
<tr>
<td><em>Citrobacter</em> (3 species)</td>
<td></td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td></td>
</tr>
<tr>
<td><em>C. diversus</em></td>
<td></td>
</tr>
<tr>
<td><em>C. amalonaticus</em></td>
<td></td>
</tr>
<tr>
<td><em>Proteus</em> (4 species)</td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em>&lt;sup&gt;d&lt;/sup&gt;</td>
<td><em>C. koseri</em></td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td><em>C. intermedius</em> biotype</td>
</tr>
<tr>
<td><em>P. penneri</em></td>
<td></td>
</tr>
</tbody>
</table>

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<sup>a</sup>Major coliforms in nosocomial and/or community-acquired human diseases (prevalent in published reports).

<sup>b</sup>Organisms previously designated as *Proteus mirabilis* and *Proteus rettgeri* are now classified in the genera *Morganella* and *Providencia*, respectively.
The coliforms

- include overt and opportunistic pathogens responsible for a wide range of infections
- Many species are members of the normal intestinal flora.
- *Escherichia coli* (*E.coli*) is the most commonly isolated organism in the clinical laboratory.
Types

- Enteric infections
- Nosocomial infections
- Community acquired infections
Structure, Classification, and Antigenic Types

- The coliforms and *Proteus* are Gram negative bacilli.
- All genera except *Klebsiella* are flagellated.
- Some strains produce capsules.
- Virulence often depends on the presence of attachment pili (which can be characterized by specific hemagglutinating reactions).
- Sex pili also may be present. The major classes of antigens used in defining strains are H (flagellar), O (somatic), and K (capsular).
Structure and antigenic composition of coliforms and *Proteus* species.
<table>
<thead>
<tr>
<th>Virulence Factor</th>
<th>Proposed Role(s) in Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Col V plasmid</td>
<td>Codes for a siderophore (aerobactin) for Fe chelation. Increases bacterial resistance to serum.</td>
</tr>
<tr>
<td>Hemolysin</td>
<td>Damages host cells. Releases Fe from red blood cells.</td>
</tr>
<tr>
<td>Enterochelin</td>
<td>Chelates Fe for bacterial uptake.</td>
</tr>
<tr>
<td>K1 antigen</td>
<td>Impedes phagocytosis. Blocks binding of C3b opsonin.</td>
</tr>
<tr>
<td>P-pili</td>
<td>Allow bacteria to bind to P blood group antigens on urinary tract cells (especially in kidneys).</td>
</tr>
<tr>
<td>Type 1 pili</td>
<td>Allow bacteria to bind to (1) bladder epithelium, (2) Tamm-Horsfall glycoprotein, and (3) D-mannose residues on a variety of cells.</td>
</tr>
</tbody>
</table>
Eschericia coli

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Eschericia coli

- naturally found in the intestines of man and animals.
- Only certain strains of E. coli are known to cause food poisoning (eg, E coli 0157).
- Illness usually within 12 to 24 hours but sometimes up to 72 hours.
- Illness caused after eating affected food.
  - caused by the bacteria multiplying in the intestine and producing toxins.
  - The illness normally lasts from one to seven days.
Contamination of food is caused by the transfer of E. coli from faeces to food.
Role in disease

- Intestinal or extraintestinal conditions
- Classification
- The enteric *E. coli* are divided on the basis of virulence
  - enterotoxigenic (ETEC),
  - enteropathogenic (EPEC);
  - enteroinvasive (EIEC),
  - verotoxigenic (VTEC)
  - enterohaemorrhagic (EHEC)
  - enteroaggregative *E. coli* (EAggEC, )
Major causes of urinary tract infections.
Pathogenic *Escherichia coli*

<table>
<thead>
<tr>
<th>Abbrev.</th>
<th>Full name</th>
<th>Common name and features</th>
<th>inoculum</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETEC</td>
<td>Enterotoxin <em>E. coli</em></td>
<td>Montizuma’s revenge, traveler’s tummy</td>
<td>$10^8$</td>
<td>Faecal contamination</td>
</tr>
<tr>
<td>EIEC</td>
<td>Enteroinvasive <em>E. coli</em></td>
<td>Invades, <em>Shigella</em> pathogenicity island</td>
<td>high</td>
<td>Food &amp; waterborne</td>
</tr>
<tr>
<td>EPEC</td>
<td>Enteropathogenic <em>E. coli</em></td>
<td>Pedestal formation, infant diarrhoea</td>
<td>$10^8-10$</td>
<td>Nosocomial, community</td>
</tr>
<tr>
<td>EHEC</td>
<td>Enterohaemorrhagic <em>E. coli</em></td>
<td>(O157)“Hamburger disease” Shiga toxin</td>
<td>3</td>
<td>Cattle faeces, meat</td>
</tr>
</tbody>
</table>
Antibiotic therapy

• Appropriate
  – guided by laboratory analysis of the antibiotic sensitivities
  – *E. coli* are resistant to many antibiotics used against Gram-positive organisms.

• Newer technologies
Strains

• particular characteristics that make it distinguishable from other *E. coli* strains
• often detectable only on the molecular level
• may result in changes to the physiology or lifecycle of the bacterium, for example leading to pathogenicity
• Different strains of *E. coli* live in different kinds of animals,
Role in microbiology

- Ubiquity
- "workhorse" in molecular biology
- Strains are adapted to lab environments
- Model to study Bacterial conjugation
- Biological engineering
- Factories
Role in water purification and sewage treatment

• The presence of coliform bacteria in surface water is a common indicator of faecal contamination.
• *E. coli* is commonly used as a model organism for bacteria in general. This is usually done using the MPN (most probable number) tests.
• "Presence" of *E. coli* numbers beyond a certain cut-off indicates faecal contamination of water and indicates further investigation into the matter.
• In the field of water purification and sewage treatment, *E. coli* was chosen very early in the development of the technology as an "indicator" of the pollution level of water.
Salmonella spp.
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Salmonellae

• human and animal pathogens

• Salmonellosis in humans
  – usually takes the form of a self-limiting food poisoning (gastroenteritis), but
  – occasionally manifests as a serious systemic infection (enteric fever) which requires prompt antibiotic treatment.

• In addition, salmonellosis causes substantial losses of livestock.
Salmonellae

- Gram-negative,
- Flagellated,
- Facultatively anaerobic bacilli
- Possessing three major antigens:
  - H or flagellar antigen;
  - O or somatic antigen; and
  - Vi antigen (possessed by only a few serovars).
Salmonellae

• Lipopolysaccharide (LPS)
  – liberated on lysis of the cell and, to some extent, during culture.
  – May function as an endotoxin,
  – may be important in determining virulence of the organisms.

• Lipopolysaccharide structure is important for several reasons.
  – First, the nature of the repeating sugar units in the outer O-polysaccharide chains is responsible for O antigen specificity;
  – antibodies directed against the R core (common enterobacterial antigen) may protect against infection by a wide variety of Gram-negative bacteria sharing a common core structure or may moderate their lethal effects.
  – Third, the endotoxin component of the cell wall may play an important role in the pathogenesis of many clinical manifestations of Gram-negative infections
Pathogenesis

1. Entry

2. Spread
   (infrequent, typhoid fever)

3. Disease
   Gastroenteritis
   Diarrhea

4. Exit
   (gallbladder-carrier state)
Pathogenesis of *Salmonella* enterocolitis

1. Ingestion of organisms
2. Colonization of lower intestine (ileum and cecum)
3. Mucosal invasion
4. Cytotoxin
5. Acute inflammation
   - ± ulceration
   - Prostaglandin synthesis
   - Enterotoxins
   - Cytokines
6. Activation of adenyl cyclase
7. ↑ Cyclic AMP
8. Fluid production (large and small bowel)
9. Diarrhea
Invasion of intestinal mucosa by *Salmonella*
# Host Defenses

## TABLE 21-2  Host Defenses Against Salmonellae

<table>
<thead>
<tr>
<th>Host Defense</th>
<th>Examples of Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastric Factors</strong></td>
<td></td>
</tr>
<tr>
<td>Gastric acidity</td>
<td></td>
</tr>
<tr>
<td>Rate of gastric emptying</td>
<td></td>
</tr>
<tr>
<td><strong>Intestinal Factors</strong></td>
<td></td>
</tr>
<tr>
<td>Intestinal motility</td>
<td></td>
</tr>
<tr>
<td>Normal intestinal flora</td>
<td></td>
</tr>
<tr>
<td>Mucus</td>
<td></td>
</tr>
<tr>
<td>Secretory antibodies</td>
<td></td>
</tr>
<tr>
<td>Genetic resistance to invasion</td>
<td></td>
</tr>
<tr>
<td><strong>Nonspecific and Other Possible Factors</strong></td>
<td></td>
</tr>
<tr>
<td>Nutritional state</td>
<td></td>
</tr>
<tr>
<td>Lactoferrin</td>
<td></td>
</tr>
<tr>
<td>Gut reticuloendothelial cells</td>
<td></td>
</tr>
<tr>
<td>Lysozyme</td>
<td></td>
</tr>
</tbody>
</table>
# Factors Increasing Susceptibility to Salmonellosis

<table>
<thead>
<tr>
<th>Location or Factor</th>
<th>Specific Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>Achlorhydia</td>
</tr>
<tr>
<td></td>
<td>Gastric Surgery</td>
</tr>
<tr>
<td>Intestine</td>
<td>Antibiotic administration</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal surgery</td>
</tr>
<tr>
<td></td>
<td>? Idiopathic inflammatory bowel disease</td>
</tr>
<tr>
<td>Hemolytic Anemias</td>
<td>Especially sickle cell anemia and other hemoglobinopathies</td>
</tr>
<tr>
<td>Impaired Systemic Immunity</td>
<td>Carcinomatosis, leukemias, lymphomas</td>
</tr>
<tr>
<td></td>
<td>Diabetes mellitus, Immunosuppressive drugs, acquired immunodeficiency syndrome (AIDS), etc</td>
</tr>
</tbody>
</table>
Control

- Difficult to eradicate from the environment
- The major reservoir for human infection is poultry and livestock,
- Control via
  - Feed treatment
  - Changing animal slaughter practices
  - Protecting processed foods
  - Training in hygienic practices
  - Cooking and refrigeration of foods
  - Expansion of disease surveillance systems