Emerging Food-Borne Bacterial Pathogens

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Food borne diseases result from ingestion of a wide variety of foods contaminated with pathogenic microorganisms, microbial toxins, or chemicals.
• Actual incidence unknown, estimated app./ year
  
  – 76 million cases,
  – 325,000 hospitalizations,
  – 5000 deaths.
  

• From 1993-97, **550 outbreaks**, affected **17,000** persons in US.
  
  (MMWR 2000;49:1-51)

• In 2005 16,614 cases were reported, **205 outbreaks** and **159** linked to restaurants.
  
  (MMWR, 2006,55:392)
What are emerging pathogens?

1. Infectious diseases whose incidence has increased in the past 2 decades or threatens to increase in the near future

2. New infections resulting from changes or evolution of existing organisms

3. Known infections spreading to new geographic areas or populations
What are emerging pathogens?

4. Old infections reemerging as a result of their appearing in new vehicles

5. Previously unrecognized infections

6. Organisms on which to keep a watchful eye
Factors in the Emergence of Pathogens

- Age
- HIV
- Malnutrition

Human Host

Pathogen

Exposure

Foodborne Illness

Adapted from: IFT, 2002. Emerging Microbiological Food Safety Issues. Implications for control in the 21st century
Factors in the Emergence of Pathogens

- Microbial adaptation and change
- Human susceptibility to infection
- Climate and weather
- Changing ecosystems
- Human demographics and behaviour
- Economic development and land use
- International travel and commerce
- Technology and industry
- Breakdown of public health measures
- Poverty and social inequality
- War and famine
- Lack of political will
- Intent to Harm
Bacterial agents

- **B.cereus**
- **Botulism, food borne**
- **Brucella spp.**
- **Campylobacter spp**
- **C.perfringens**
- **E.coli O157:H7**
- **E.coli, non – O157 STEC**
- **E.coli, enterotoxigenic**
- **E.coli, other diarrheogenic**
- **Listeria monocytogens**

- **S.typhi**
- **Salmonella, nontyphoidal**
- **Shigella spp**
- **Staphylococcal food poisoning**
- **Streptococcus, foodborne**
- **V.cholerae, toxigenic**
- **V.vulnificus**
- **Other Vibrios**
- **Y.enterocolitica**
- **Y.pseudotuberculosis O3**

(Red- emerging pathogens)
- Parasitic agents
  - *C. parvum*
  - *C. cayatanensis*
  - *G. lamblia*
  - *T. gonodii*
  - *T. spiralis*
  - *E. histolytica*

- Viral agents
  - *Norwalk viruses*
  - *Rotavirus*
  - *Astrovirus*
  - *Hepatitis A virus*
  - *Hepatitis E virus*
  - *Influenza*

- Metazoan parasites
  - Round worm
  - Tapeworm
  - Flukes

- Fungi
  - *Aspergillus*
  - *Fusarium*
  - *Penicillium*
# Pathogenic Mechanism

<table>
<thead>
<tr>
<th>Preformed Toxin</th>
<th>Toxin production</th>
<th>Tissue invasion</th>
<th>Toxin / Tissue Invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>C. perfringens</td>
<td>C. jejuni</td>
<td>V. parahemolyticus</td>
</tr>
<tr>
<td>B. cereus (SI)</td>
<td>B. cereus (LI)</td>
<td>Salmonella</td>
<td>Y. enterocolitica</td>
</tr>
<tr>
<td>C. botulinum</td>
<td>C. botulinum</td>
<td>Shigella</td>
<td></td>
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<tr>
<td></td>
<td>(infant botulism)</td>
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<tr>
<td></td>
<td>Enterotoxigenic E. coli</td>
<td>Invasive E. coli</td>
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<tr>
<td>V. cholerae O1 /O139</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>V. cholerae non-O1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>STEC</td>
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</tbody>
</table>
Nontyphoidal Salmonellosis

- **Source:** Eggs, Poultry, Undercooked meat, Unpasteurized dairy products, sea food.

- **Incubation period:** 6-48 hours

- **The serotypes are:** Typhimurium (19%)  
  Enteritidis (18%)  
  Newport (10%)  
  Heidelberg (6%)

- **Antibiotic Resistance:**  
  *S. typhimurium* DT104- resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines.
**Campylobacter**

- **Incidence:**
  - 2.5 million cases per year (CDC estimate/NIAID)

- They are found in GIT of many animals used for food (poultry, cattle, sheep, and swine) and of many household pets (birds, dogs and cats).

- They do not cause illness in their animal hosts.

- **Source:** raw or undercooked food or direct contact with infected animals, unpasteurized milk.

- **Antibiotic resistance:** Resistance to ciprofloxacin, tetracycline, erythromycin have been reported.
NEWER VEHICLE

• Outbreak of Salmonella Serotype Hartford Infections Associated With Unpasteurized Orange Juice.

• Acidic foods such as orange juice have been thought to be unlikely vehicles of food borne illness.

• All orange juice was recalled and the processing plant closed.

• Pasteurization or other equally effective risk-management strategies should be used in the production of all juices.

(Kim A. Cook et al, JAMA, November 4, 1998—Vol 280, No. 17)
Detection of Campylobacter and Salmonella in Turkey semen

- The contaminated pooled semen spread bacteria throughout entire flocks of hen via artificial insemination.

- Results demonstrate that semen extenders tested did not eliminate these bacteria from semen.

- Hence concluded that semen may be a potential vehicle for Salmonella and Campylobacter transfer to hens.

An International Outbreak of Salmonellosis Associated with Raw Almonds

• During 2000-01, an outbreak due to *Salmonella enteritidis* (SE) phage type 30 (PT30), a rare strain, was detected in Canada.

• The case-control study was conducted to test the hypothesis of an association between raw whole almond consumption and infection.

• This was previously undocumented finding, leading to recall of this product and a review of current industry practices.

Insight of Resistance

- *S. typhimurium* phage type **DT104** is the most prevalent multiresistant / hypervirulent strain.

- Multiresistance in DT104 is conferred by an integron structure, *Salmonella* genomic island 1 (SGI1).

  (Steve A. Carlson et al., INFECTION AND IMMUNITY, Feb. 2007, p. 792–800)

- The detection of **DT104** and **DT193** in shellfish emphasizes monitoring the presence of *Salmonella* in routine surveillance of live bivalve molluscs.

- Two DT104 isolates and a PT 193 were resistant to multiple antibiotics.

  (Jaime Martinez-Urtaza et al., APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2004, p. 4030–4034)
Protozoan *Acanthamoeba polyphaga* as Potential Reservoir for *Campylobacter jejuni*

- The four different *Campylobacter jejuni* strains infect the protozoan *Acanthamoeba polyphaga*.
- *C. jejuni* cells survived for longer periods when cocultured with amoebae.
- A resuscitation of bacterial cultures was observed after reinoculation into fresh amoeba cultures.
- Amoebae may serve as a nonvertebrate reservoir for *C. jejuni* in the environment.

Emerging Pathogens

2. New infections resulting from changes or evolution of existing organisms
Escherichia coli

- Shiga-toxin producing *E. coli* (STEC)
- Source: undercooked ground beef, manure from cattle or other animals can contaminate produce (potatoes, lettuce, sprouts, fallen apples).
- Incubation period: 3-4 days.
- Hemorrhagic colitis and the hemolytic uremic syndrome (HUS)
- O 157:H7 is the most prominent serotype.
- Other pathotypes of *E. coli* are acquired predominantly through fecal-oral route.
Isolation of shiga toxin producing *E. coli* from dairy cattle and beef, Calcutta

- The study provided the first evidence that dairy cattle in Calcutta is the potential reservoir for virulent O157:H7 strain.
- Also identified that beef marketed in Calcutta, as a source for virulent O157:H7 strain.
- The isolation of eaeA and hyl A positive O157:H7 strains from meat reflected it as pathogenic strain.
- No STEC was isolated from diarrhoea cases.
- The low prevalence of STEC associated diarrhoea in India may be due to cooking practices and small proportion of population that consume beef.

Non- O 157 *E. coli* Outbreaks

- **O111**- 1994-2000 - USA, Canada
  - Ground beef, unpasteurized apple, salad.

- **O129**- 1999-2006- USA, Canada
  - Lettuce, lake water.

- Other emerging are **O26, O103, O45**.
Use of Copper Cast Alloys To Control *E. coli* O157 Cross-Contamination during Food Processing

- The results demonstrated the antimicrobial properties of cast copper alloys with regard to *E. coli* O157, and consequently these alloys have the potential to aid in food safety.

- With beef juice, only one alloy (95% Cu) completely killed the inoculum at 22°C.

- For stainless steel, no significant reduction in cell numbers occurred.

**Clostridium perfringens**

- **Type A** is the most common cause of food poisoning.
- The cytotoxin is produced by >75% of strains isolated from cases of food borne disease.
- **Source**: meat, meat products and poultry.
- **Outbreaks**: due to problem in cooling and storage of food cooked in bulk.
- During reheating, they sporulate and germinate.
- **Incubation period**: 8-24 hours.
**Clostridium botulinum**

- **Food borne botulism**: from ingestion of preformed toxin in food contaminated with *C. botulinum*.
- **Intestinal botulism**: from ingestion of spores and production of toxin in intestine of infants (infant botulism) or adults.
- Toxin A, B, E and rarely F cause human disease.
- **Source**: home canned food - vegetables, fruit, condiments, meat and fish.
- **Incubation period**: 18-36 hours.
Botulism Associated With Commercially Canned Chili Sauce-Texas and Indiana, July 2007

- On July 7 and 11, 2007, public health officials in Texas and Indiana, reported to CDC four suspected cases of food borne botulism, two in each state.

- Investigations conducted revealed that all four patients had eaten brands of Castleberry's hot dog chili sauce before illness began.

- **Toxin type A** was detected in the serum of one Indiana patient and in a leftover chili mixture obtained from his home.

- The manufacturer, recalled the implicated brand.

*(JAMA. 2007;298:1154-1156)*
Clostridium difficile in Retail Ground Meat, Canada

• Abstract

Clostridium difficile was isolated from 12 (20%) of 60 retail ground meat samples purchased over a 10-month period in 2005 in Canada. Eleven isolates were toxigenic, and 8 (67%) were classified as toxinotype III. The human health implications of this finding are unclear, but with the virulence of toxinotype III strains further studies are required.

(Alexander Rodriguez-Palacios et al, EID Volume 13, Number 3–March 2007)
Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans

- From May 2001 to April 2003, various types of specimens from cattle, pigs, and chickens were collected and examined for the presence of MRSA.

- The data suggested that the genomes of the six animal MRSA isolates were very closely related to those of some human MRSA isolates and were a possible source of human infections caused by consuming contaminated food products made from these animals.

An Outbreak of *Yersinia pseudotuberculosis* O3 Infections Associated with Consumption of Iceberg Lettuce in Finland

- The source for *Y. pseudotuberculosis* (YP) infections has remained unknown in previous outbreaks.

- In October 1998, reported YP, serotype O3 (YP-O3) infections increased markedly.

- Case-patients were more likely than controls to report having eaten iceberg lettuce in the 2 weeks before illness onset (71% vs. 42%; matched OR, 3.9; 95% CI, 1.3-9.6).

- No other fresh produce, or meat product was associated with illness.

**Vibrio parahaemolyticus**

- Infections with this organism have been associated with the consumption of raw, improperly cooked, or cooked, recontaminated fish and shellfish.

  
  *(MMWR: 47,1997)*


  *(MMWR: 48,1998)*
• This highlights the need for enhanced surveillance for human infections.

• PCR approach used to monitor the occurrence and distribution of this newly emerged pathogenic *V. parahaemolyticus O3:K6* strain in coastal, marine, and ship ballast waters.

(Michael L. Myers et al, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2003, p. 2194–2200)
Viable but non cultivable Vibrios

- Human volunteer studies suggested that VBNC *V. cholerae* maintains pathogenic potential in the environment with human passage effectively triggering an outgrowth of the cells to the viable and cultivable state


- In such scenario we need to have Molecular tools which can explore this state of organisms
Food Net

• The Food borne Diseases Active Surveillance Network (FoodNet) of CDC’s Emerging Infections Program collects data from 10 U.S. states regarding diseases caused by enteric pathogens transmitted commonly through food.

• Food-Net quantifies and monitors the incidence of these infections by conducting active, population-based surveillance for laboratory-confirmed illness.
In 1996

- Active surveillance began for laboratory-confirmed cases of
  
  *Campylobacter*
  *Escherichia coli* O157
  *Listeria monocytogenes*
  *Salmonella*
  *Shigella*
  *Vibrio*
  *Yersinia entercolitica* infections

In 1997

- Laboratory-confirmed cases of *Cryptosporidium* spp. and *Cyclospora cayetanensis* infections was added

In 2000,

- Data on *STEC* -O157 and comprehensive information on hemolytic uremic syndrome (HUS) was added.
The data indicate

• The relative frequency of diagnosed infections.
• Demonstrate substantial regional variation.
• Suggest trends in incidence.

FoodNet provides data for

• monitoring food borne illnesses.
• Interventions designed to reduce them.

In 5 years (1996-2000) of FoodNet data collection,

• Campylobacter was the most frequently diagnosed pathogen.
• Followed by Salmonella, Shigella, and E. coli O157.
• However, substantial regional and year to year variation occurred.
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<tr>
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<tbody>
<tr>
<td>Campylobacter</td>
<td>23.5</td>
<td>22.4</td>
<td>12.72</td>
<td>12.3</td>
</tr>
<tr>
<td>Salmonella</td>
<td>14.5</td>
<td>13.45</td>
<td>14.55</td>
<td>6.8</td>
</tr>
<tr>
<td>Shigella</td>
<td>8.9</td>
<td>9.95</td>
<td>4.61</td>
<td></td>
</tr>
<tr>
<td>E.Coli O:157</td>
<td>2.7</td>
<td>2.55</td>
<td>1.06</td>
<td>1.0</td>
</tr>
<tr>
<td>Yersinia</td>
<td>1.0</td>
<td>0.55</td>
<td>0.36</td>
<td></td>
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<tr>
<td>Listeria</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.25</td>
</tr>
<tr>
<td>Vibrio</td>
<td>0.1</td>
<td>0.45</td>
<td>0.27</td>
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</table>

MMWR
Comparing incidence of two Salmonella Organisms 1996 2000

<table>
<thead>
<tr>
<th>Organisms</th>
<th>1996</th>
<th>2000</th>
</tr>
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<tbody>
<tr>
<td><em>Salmonella typhi</em></td>
<td>3.9%</td>
<td>2.7%</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>2.5%</td>
<td>1.8%</td>
</tr>
</tbody>
</table>
Noroviruses

Are Noroviruses Emerging?

Marc-Alain Widdowson,* Stephan S. Monroe,* and Roger I. Glass†

Four outbreaks of norovirus gastroenteritis after consuming raspberries, Sweden, June-August 2006
Rotaviruses

- Acute gastroenteritis in children/infants
- Faeco-oral transmission, highly infective
- Global burden estimate: 138 million infections, over 600,000 deaths each year
- Severe disease preventable by live attenuated oral vaccines
- Vaccination programs being evaluated in Europe
- Found in raw retail meats
Emerging Parasites

• *Cryptosporidium* *spp.* 2.95/100000 population reported in 2005.
  – Large outbreaks associated with visit to recreational water park in New York, 2004-05.

• *Cyclospora*
  – Atlanta GA, May 2000, 21 cases
    • raspberries (Guatemala)
  – Florida, Mar-Apr 2005, 293 cases
    • Basil (Peru)
  – Vancouver BC, Jun-Jul 2006, 14 cases
    • basil / garlic?
Molecular methods

PCR for the early & correct detection of Salmonella serotypes in food/feces & blood

Salmonella – PCR [ Genus specific ]

(R. Chaudhry et al, Medical Journal of Indonesia, suppl-1998)
**Clostridium perfringens**

- Identification of *C. perfringens* using phospholipase C gene and enterotoxin gene by PCR

**Advantages:**
- In vitro sporulation is difficult - enterotoxin detection Molecular level identification better

![Image of gel electrophoresis result]

- **M** - marker (100 Bprs)
- Lane 1 to 8 - Non enterotoxigenic *C. perfringens*
- Lane 9 - Enterotoxigenic *C. perfringens*
**Clostridium botulinum** neurotoxin gene

- **Lane M**: Marker (100-1000 bp)
- **Lane 1**: *C. botulinum* type A (782 bp)
- **Lane 2**: *C. botulinum* type B (205 bp)
- **Lane 3**: *C. botulinum* type E (389 bp)
- **Lane 4**: *C. botulinum* type F (543 bp)
- **Lane 5**: Negative control
- **Lane 6**: *C. botulinum* types A, B, E & F
• Such active surveillance network needs to be established in each country to have true global burden of food borne pathogens so as appropriate interventions can be implemented at an early phase.

• The formulation of internationally acceptable standards for food premises and of public health infrastructure would be of great value.
Evolving and adapting foodborne pathogens in a backdrop of environmental and behavioural changes provide new ecological niches into which evolving microbes can easily fit and prosper, leading to emerging and reemerging food borne pathogens.
THANK YOU

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