Microbiological food safety challenges in the Indian dairy industry

Theme:
Session 2: Microbiological Sampling and Testing: Food Safety Management

FSSAI-ICMSF-CHIFSS Symposium On Microbiological Food Safety Sampling And Testing In Food Safety Management

Delivered By:
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Emergence of microbiological food safety concerns and challenges

Major trends

- Increased awareness among consumers on food safety and quality
- Food consumption patterns: minimal processed foods, preference for processed ready to eat foods
- Use of new additives /ingredients and development of new products
- Shift in agricultural production, manufacturing and distribution practices in food chain
- International travel and trade - transportation of infectious agents
- Globalization of food supply chain
- Detection, reporting and surveillance system
- Acquisition of virulence and antibiotic genes by pathogenic bacteria - Emergence of antimicrobial resistance in bacteria (AMR)
- Microbial adaptation and enhanced survival of pathogens in food
Risk profiling of pathogens in milk and milk Products

<table>
<thead>
<tr>
<th>Organism</th>
<th>Shed directly in milk#</th>
<th>Contaminant of raw milk##</th>
<th>Survives pasteurization</th>
<th>Severity of illness §</th>
<th>Dairy products implicated in food-borne illness</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas</em> spp.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td><em>Brucella</em> spp.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Severe</td>
<td>+</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>✓</td>
<td>✓</td>
<td>*</td>
<td>Severe</td>
<td>+</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Moderate</td>
<td>+</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td><em>Coxiella burnetii</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Severe</td>
<td>+</td>
</tr>
<tr>
<td><em>Mycobacterium avium</em> subs. <em>paratuberculosis</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Mycobacterium bovis</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Severe</td>
<td>+</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serious</td>
<td>+</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Moderate</td>
<td>++</td>
</tr>
<tr>
<td><em>Staphylococcus</em> aureus</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Moderate</td>
<td>++</td>
</tr>
<tr>
<td><em>Campylobacter jejuni / coli</em></td>
<td>✓</td>
<td>✓</td>
<td>✓**</td>
<td>Moderate</td>
<td>++</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serious</td>
<td>++</td>
</tr>
<tr>
<td><em>Enterobacter sakazakii</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Severe^</td>
<td>++</td>
</tr>
<tr>
<td><em>Pathogenic E. coli</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Severe</td>
<td>++</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Severe^</td>
<td>++</td>
</tr>
</tbody>
</table>

Transmission through udder; mastitis etc; ## via faeces, the environment etc; *Neurotoxin is heat labile; ** Enterotoxin is heat stable; ^ for vulnerable populations; § based on ICMSF (2002) severity ranking; + Reported, but rare; ++ More commonly associated with food-borne illness; − No data/unknown; ✓ = yes; X = no
Outbreaks associated with dairy products reported in US: 2000-2013

- Campylobacter: 74%
- E. coli O157:H7: 5%
- Norovirus: 5%
- Salmonella: 4%
- Listeria: 3%
- Staphylococcus: 1%

EU Report on Food borne Outbreaks -2011

- Campylobacter: 66%
- Salmonella: 29%
- E. coli O157:H7: 3%
- Yersinia: 2%
- Listeria: 0%
- Mycobacterium: 0%

Raw milk, Pasteurized milk, Cheeses and Powdered milk

www.realrawmilkfacts.com

10/10/2018
Microbiological food safety challenges in the Indian dairy industry
COMMON SOURCES OF FOOD POISONING

Estimated total illnesses from outbreaks in 1998-2012

- Salmonella
- E. Coli
- Campylobacter
- Listeria

- Beef
- Pork
- Chicken
- Turkey
- Other meat
- Seafood
- Dairy
- Eggs
- Vegetables
- Fruits
- Grains/Beans
- Sprouts
- Other produce

*Includes estimated total illnesses for only outbreaks that could be attributed to a single pathogen and food category

Source: Centre for Disease control and prevention
<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Cases</th>
<th>Product</th>
<th>Causative agent</th>
<th>Cause</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>USA</td>
<td>13</td>
<td>Raw milk</td>
<td><em>C. jejuni</em></td>
<td>Unpasteurised milk</td>
<td>(Peterson, 2003)</td>
</tr>
<tr>
<td>2000</td>
<td>Austria</td>
<td>38</td>
<td>Unpasteurized milk</td>
<td><em>C. jejuni</em></td>
<td>Unpasteurised milk distributed by a local dairy</td>
<td>(Lehner et al., 2000)</td>
</tr>
<tr>
<td>1998</td>
<td>Hungary</td>
<td>52</td>
<td>Raw milk</td>
<td><em>C. jejuni</em></td>
<td>Unpasteurised milk</td>
<td>(Kalman et al., 2000)</td>
</tr>
<tr>
<td>1996</td>
<td>UK</td>
<td>33</td>
<td>Unpasteurized milk</td>
<td><em>C. jejuni</em></td>
<td>Educational farm visit, exposure to raw milk</td>
<td>(Evans et al., 1996)</td>
</tr>
<tr>
<td>1992</td>
<td>USA</td>
<td>50</td>
<td>Raw milk</td>
<td><em>C. jejuni</em></td>
<td>Consumed at church</td>
<td>(CDC 2002)</td>
</tr>
<tr>
<td>1995</td>
<td>UK</td>
<td>110</td>
<td>milk</td>
<td><em>C. jejuni</em></td>
<td>Inadequately pasteurized milk from a local dairy</td>
<td>(Fahey et al., 1995)</td>
</tr>
</tbody>
</table>
### Major Outbreaks associated with *Salmonella* spp.

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Cases</th>
<th>Product</th>
<th>Causative agent</th>
<th>Cause</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>USA</td>
<td>38</td>
<td>Pasteurized milk</td>
<td><em>Salmonella typhimurium</em></td>
<td>Likely contaminated containers or milk contact surfaces after pasteurisation because of environmental conditions in plant</td>
<td>(Olsen <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>2003</td>
<td>USA</td>
<td>62</td>
<td>Raw milk</td>
<td><em>Salmonella typhimurium</em></td>
<td>Unpasteurised milk at dairy/petting zoo</td>
<td>(Mazurek <em>et al.</em>, 2004)</td>
</tr>
<tr>
<td>1998</td>
<td>UK</td>
<td>40</td>
<td>Pasteurized milk</td>
<td><em>Salmonella</em></td>
<td>Pasteurisation failure</td>
<td>(Brown, 1998)</td>
</tr>
<tr>
<td>1999</td>
<td>Washing</td>
<td>17</td>
<td>Raw-Milk Cheese</td>
<td><em>Salmonella typhimurium</em> DT 104</td>
<td>Infection due to consumption of unpasteurised milk</td>
<td>(Villar <em>et al.</em>, 1999)</td>
</tr>
</tbody>
</table>

Microbiological food safety challenges in the Indian dairy industry
## Outbreaks associated with *Listeria monocytogenes*

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Reported cases</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PASTEURIZED MILK</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1986</td>
<td>Austria</td>
<td>28</td>
<td>Consumption of raw milk</td>
</tr>
<tr>
<td>2006-07</td>
<td>USA</td>
<td>5</td>
<td>At the plant where the milk was processed, inspections revealed no evidence of improper pasteurization</td>
</tr>
<tr>
<td>1997</td>
<td>USA</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td><strong>CHEESE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>France</td>
<td>18</td>
<td>Cheese was made from the Un-pasteurised milk / Environmental contamination</td>
</tr>
<tr>
<td>2003</td>
<td>Sweden</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>Canada</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Soft cheese</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>USA</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>Switzerland</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>USA</td>
<td>22</td>
<td>Ricotta Salata Cheese</td>
</tr>
<tr>
<td>2014</td>
<td>USA</td>
<td>5</td>
<td>Soft cheese made from pasteurized milk</td>
</tr>
<tr>
<td><strong>BUTTER AND BUTTER PRODUCT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>UK</td>
<td>17</td>
<td>Listeria isolated from a dairy drain / butter</td>
</tr>
</tbody>
</table>
Pathogenic bacteria isolated from different Indian foods

<table>
<thead>
<tr>
<th>BFJ 114,5</th>
<th>Type of food</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk</td>
<td><em>Listeria monocytogenes, Yersinia enterocolitica, Bacillus cereus, Staphylococcus faecalis, Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td>Meat</td>
<td><em>Bacillus cereus, Escherichia coli Staphylococcus aureus, Vibrio parahaemolyticus</em></td>
</tr>
<tr>
<td></td>
<td>Beef sample</td>
<td><em>Escherichia coli 0157:H7</em></td>
</tr>
<tr>
<td></td>
<td>Sweets</td>
<td><em>Salmonella Newport, Salmonella enteritidis</em></td>
</tr>
<tr>
<td></td>
<td>Dahi (yogurt), Khoa</td>
<td><em>Escherichia coli, Enterobacter aerogenes Salmonella Newport, Salmonella enteritidis, Fecal coliforms</em></td>
</tr>
<tr>
<td></td>
<td>Prawns</td>
<td><em>Vibrio parahaemolyticus</em></td>
</tr>
<tr>
<td></td>
<td>Cooked and uncooked rice</td>
<td><em>Bacillus cereus</em></td>
</tr>
<tr>
<td></td>
<td>Poultry</td>
<td><em>Campylobacter jejuni, Salmonella bornum</em></td>
</tr>
<tr>
<td></td>
<td>Fish</td>
<td><em>Staphylococcus, Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td>Samosa</td>
<td><em>S.aureus</em></td>
</tr>
<tr>
<td></td>
<td>Batatawada</td>
<td><em>S.aureus</em></td>
</tr>
<tr>
<td></td>
<td>Tamarind</td>
<td><em>Salmonella, Staphylococcus, Shigella</em></td>
</tr>
<tr>
<td></td>
<td>Butter milk</td>
<td><em>Yersina enterocolitica</em></td>
</tr>
</tbody>
</table>

Source: Vemula et al., 2010
# Common foodborne pathogens and their percentage of contamination in Indian foods

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Bacteria</th>
<th>% of contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td><em>B. cereus</em></td>
<td>16-50</td>
</tr>
<tr>
<td></td>
<td><em>L. monocytogenes</em></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>Yersinia</em></td>
<td>5-50</td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas</em></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio</em></td>
<td>8</td>
</tr>
<tr>
<td>Meat</td>
<td><em>Salmonella spp</em></td>
<td>3-5</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus spp</em></td>
<td>21</td>
</tr>
<tr>
<td></td>
<td><em>E.coli</em></td>
<td>9-14</td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas</em></td>
<td>13</td>
</tr>
<tr>
<td></td>
<td><em>B. cereus</em></td>
<td>35</td>
</tr>
<tr>
<td>Poultry</td>
<td><em>C. jejuni</em></td>
<td>41</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella spp</em></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas</em></td>
<td>28</td>
</tr>
<tr>
<td>Fish</td>
<td><em>E.coli</em></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio</em></td>
<td>16-32</td>
</tr>
<tr>
<td></td>
<td><em>Shigella</em></td>
<td>4</td>
</tr>
<tr>
<td>Seafoods</td>
<td><em>Salmonella spp</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio spp</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Listeria</em></td>
<td>1</td>
</tr>
<tr>
<td>Beef</td>
<td><em>E.coli 0157:H7</em></td>
<td>60</td>
</tr>
<tr>
<td>Rice</td>
<td><em>B. cereus</em></td>
<td>28-46</td>
</tr>
<tr>
<td>Vegetables</td>
<td><em>B. cereus</em></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td><em>Coliforms</em></td>
<td>75</td>
</tr>
<tr>
<td></td>
<td><em>E.coli</em></td>
<td>75</td>
</tr>
<tr>
<td></td>
<td><em>Listeria</em></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td><em>C. jejuni</em></td>
<td>3</td>
</tr>
<tr>
<td>Lassi</td>
<td><em>B. cereus</em></td>
<td>5</td>
</tr>
<tr>
<td>Khoa</td>
<td><em>Staphylococcus spp</em></td>
<td>20-36</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella spp</em></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>E.coli</em></td>
<td>9</td>
</tr>
</tbody>
</table>

Source: Vemula et al., 2010
Two stage enzyme assay for rapid detection of *L. monocytogenes* in milk

**Stage 1: Presumptive detection of *Listeria* spp.**

- Inoculate 25 mL of sample in LSEM and incubate at 37°C
- Observe color change from yellow to black

**Stage 2: Confirmatory detection of *L. monocytogenes***

- Green color within 4.30 h of incubation confirms *L. monocytogenes*
- Yellow color within 2.30 h of incubation further indicates the presence of genus *Listeria* spp.

**Results Validated with ISO procedure**

Conventional method - ISO: 11290 Part-1:1996 (5-7 days Protocol)

**% Incidence of *Listeria monocytogenes*** in raw and pasteurized milk samples:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>% Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>5.88</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td>1.35</td>
</tr>
<tr>
<td>Average Incidence</td>
<td>3.62</td>
</tr>
</tbody>
</table>

N=227 (R-137, P-90)

Two stage enzyme assay for detection of *E. coli* in milk

**Stage-1 Presumptive detection of *E.coli* in milk**

- Lyophilized ESM tube
- Reconstitute tube with 0.9 mL of sterile water
- Aseptically add 0.1 mL of milk sample
- Incubate at 37±1 °C for 12 ±1 h
- Blue color indicates presence of *E.coli*

**Stage-2 Confirmatory detection of *E.coli* in milk**

- Centrifuge and wash pellet twice with PB
- Wipe the tubes with sterile cotton swab
- Reconstitute lyophilized ESM tube and transfer the content to washed pellet
- Incubate at 37±1 °C for 3.0 ± 0.15h
- Blue color confirms presence of *E.coli* in milk samples

**Results Validated with ISO procedure**

- Conventional method - IS:5887 Part-I:1976 (4-5 days Protocol)
Rapid detection of coliform in milk

1. Lyophilized CSM tube
2. Reconstitute with 0.9 mL of sterile water
3. Aseptically add 0.1 mL of milk sample
4. Incubate at 37±1 °C for 12±1h
5. Yellow color confirm coliform

Results Validated with ISO procedure


Incidence of coliform in raw and pasteurized milk samples N=227 (R-137, P-90)

<table>
<thead>
<tr>
<th></th>
<th>% Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>31.26</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td>16.23</td>
</tr>
<tr>
<td>Average Incidence</td>
<td>23.75</td>
</tr>
</tbody>
</table>
Two stage enzyme assay for *Enterococci* in milk

**Stage 1: Presumptive detection of *Enterococci* in milk**

1. **From stage 1** - Inoculate 300 μl of cell suspension after centrifugation (in duplicate) into T-1 & T-2

2. **Reconstitute with 0.9 mL of sterile water**
3. **Aseptically add 100 μL of milk sample**
4. **Appearance of black color**

5. **Incubate T-1 & T-2 at 37±1°C for 3.30±0.30 h**

6. **Appearance of yellow color**

7. **Add 2-3 drops of lyophilized reagent after reconstitution in T2**

8. **Appearance of orange red color**

**Stage 2: Confirmatory detection of *Enterococci* in milk**

- **Appearance of yellow color**
- **Appearance of orange red color**

**Incidence of *Enterococci* in raw and pasteurized milk samples**

<table>
<thead>
<tr>
<th>% Incidence</th>
<th>Raw milk</th>
<th>Pasteurized milk</th>
<th>Average Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38.55</td>
<td>17.66</td>
<td>28.11</td>
</tr>
</tbody>
</table>

**Results Validated with ISO procedure**

- **IP Status: Patent Reg No 119/DEL/2012**
- **Conventional method : IS 5887 Part-2: 1976 (3-4 days Protocol)**
Incidence of Aflatoxin M1 in Milk samples

% incidence of Aflatoxin M1 in Raw Milk

- <0.25 / Negative: 87.60%
- 0.25 ppb: 5.71%
- 0.5 ppb: 6.69%
- N=508

Source: Outreach project 2010-16

% incidence of Aflatoxin M1 in Pasteurized Milk

- <0.25 / Negative: 81.25%
- 0.25 ppb: 8.25%
- 0.5 ppb: 10.5%
- N=304

Aflatoxin M1 concentration in ppb

Microbiological food safety challenges in the Indian dairy industry
Risk profiling of dairy pathogens: ICMSF 2002

Classification on basis of dose response

**Severe**
- *Listeria monocytogenes*
- Pathogenic *E. coli*
- *Brucella*, *Enterobacter sakazakii*

**Serious**
- *Campylobacter jejuni*
- *Salmonella*
- *Yersinia enterocolitica*

**Moderate**
- *S. aureus*
- *B. cereus*

Classification on basis of exposure response

**Severe**
- Raw milk/ cheeses
- Raw milk/ Meat products, Infant milk formula

**Serious**
- Raw milk /Broiler meat products / Poultry/ Ice-cream

**Moderate**
- Khoa based sweets

**Severe:** Life threatening, or substantial sequelae, prolong duration

**Serious:** In incapacitating but not life threatening; sequelae infrequent; moderate duration

**Moderate:** Not usually life threatening; nosequelae; short duration; self-limiting; severe discomfort
FSSAI Microbiological criteria approved for Milk & milk Products

Micro-organisms

1. Total Plate Counts
2. Coliform
3. Yeast and Mold Counts
4. *Staph aureus*
5. *Bacillus cereus*
6. *E. coli*
7. *Salmonella*
8. *Listeria monocytogenes*
9. *Enterobacter sakazakii*
10. SRC

Risk Assessment
- Hazard identification
- Hazard characterization
- Exposure assessment
- Risk characterization

Risk management
- Risk evaluation
- Option assessment
- Option implementation
- Monitoring and review

Risk communication
Interactive exchange of information option concerning risks

Codex Alimentarius Risk Assessment frame work

Generation of scientific data on hygiene/ safety indicators in milk and milk products through National surveillance network Project
Mastitis and its impact in India

<table>
<thead>
<tr>
<th>Source of loss</th>
<th>Loss per cow($)</th>
<th>Percent of total(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced production</td>
<td>121.00</td>
<td>66.0</td>
</tr>
<tr>
<td>Discarded milk</td>
<td>10.45</td>
<td>5.7</td>
</tr>
<tr>
<td>Replacement cost</td>
<td>41.73</td>
<td>22.6</td>
</tr>
<tr>
<td>Extra labour</td>
<td>1.14</td>
<td>0.1</td>
</tr>
<tr>
<td>Treatment</td>
<td>7.36</td>
<td>4.1</td>
</tr>
<tr>
<td>Veterinary services</td>
<td>2.72</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>184.40</td>
<td>100</td>
</tr>
</tbody>
</table>

- In India annual losses in dairy industry due to mastitis has been reported approximately 1670 Cr / 231.2 Million USD (Jingar, et al, 2017)
Microorganisms involved in mastitis

<table>
<thead>
<tr>
<th>Environmental pathogens</th>
<th>Contagious pathogens</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli (40%)</td>
<td><em>Staphylococcus aureus</em> (40-70%)</td>
<td>Staphylococcus epidermidis (1.3%)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td><em>Streptococcus agalactiae</em> (8-10%)</td>
<td>Staphylococcus simulans (1.0%)</td>
</tr>
<tr>
<td>Arcanobacteriumpyogenes</td>
<td><em>Streptococcus dysgalactiae</em> (1.6%)</td>
<td>Staphylococcus chromogens (0.7%)</td>
</tr>
<tr>
<td>Yeast spp.</td>
<td>Corynebacterium spp.</td>
<td>Mycoplasma spp. (5-12%)</td>
</tr>
</tbody>
</table>

Microbiological culture of 74 cases of clinical mastitis

- **41.66%** E. coli
- **44.44%** Staphylococcus spp.
- **5.50%** Streptococci spp.
- **8.33%** Klebsiella spp.

Jeykumar et al., 2013
# Types of mastitis and Diagnostic methods for its identification

<table>
<thead>
<tr>
<th>Test</th>
<th>Identification of mastitis milk</th>
<th>Identification of pathogen</th>
<th>Time of detection</th>
<th>Test location</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td>California mastitis test</td>
<td>✓</td>
<td>×</td>
<td>Minutes</td>
<td>Farm</td>
<td>Fresh milk</td>
</tr>
<tr>
<td>Somatic cell count</td>
<td>✓</td>
<td>×</td>
<td>Minutes</td>
<td>Lab</td>
<td>Fresh milk</td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>✓</td>
<td>✓</td>
<td>Days</td>
<td>Lab</td>
<td>Fresh milk</td>
</tr>
<tr>
<td>ELISA</td>
<td>×</td>
<td>✓</td>
<td>Hours</td>
<td>Lab</td>
<td>Fresh, Frozen, Preserved</td>
</tr>
<tr>
<td>MULTIPLEX PCR</td>
<td>✓</td>
<td>✓</td>
<td>Hours</td>
<td>Lab</td>
<td>Fresh, Frozen, Preserved</td>
</tr>
</tbody>
</table>

Availability of diagnostic for early detection of mastitis will be a great help in sustainability of dairy farm business and controlling AMR
Global trends of antimicrobials use in livestock

- **63151** tones in 2010
- **67%** increase in 2030
- **100%** increase in BRICS countries

Large consumers of antimicrobials in animals in 2010 and 2030

*Figure 0-3: Antibiotic consumption by livestock, top ten countries 2010-2030 (projected for 2030)*

*Source: Van Boeckel et al. 2015*
Antibiotic consumption is growing rapidly...

Reddy et al., 2014

Diagnostic for Mastitis, Antimicrobial Residues and Resistant bacteria in dairy settings
# Existing diagnostic for detection of Antimicrobial Residues in milk

<table>
<thead>
<tr>
<th>Name of test</th>
<th>Concept / principle</th>
<th>Manufacturer</th>
<th>Time per test</th>
<th>Cost of the test</th>
<th>Antibiotic tested</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delvo test</td>
<td>Microbial inhibition based</td>
<td>Gist brocades/ DSM</td>
<td>2:30 min</td>
<td>Rs. 150 per test</td>
<td>Broad spectrum</td>
<td>Qualitative screening test use in few multinational companies / bigger dairy farms in India</td>
</tr>
<tr>
<td>DPA based assay</td>
<td>Microbial inhibition based / Color change</td>
<td>NDRI Karnal</td>
<td>3.0-3.15 h</td>
<td>Rs. 50 per test</td>
<td>Broad spectrum</td>
<td>Qualitative screening test (semi-quantitative test) are in use in few multinational companies / bigger dairy farms in India</td>
</tr>
<tr>
<td>Paper strip based assay</td>
<td>Spore germination / enzyme / color change</td>
<td>NDRI Karnal</td>
<td>1.00 h</td>
<td>Rs. 75 per test</td>
<td>Broad spectrum</td>
<td></td>
</tr>
<tr>
<td>Penzym Test</td>
<td>Enzyme colorimetric</td>
<td>Neogen Corporation</td>
<td>15 min</td>
<td>Rs. 200 per test</td>
<td>β-lactams</td>
<td>Not in use</td>
</tr>
<tr>
<td>Tetra sensor</td>
<td>Antigen antibody based</td>
<td>Different companies</td>
<td>5-10 min</td>
<td>~Rs. 200 for one antibiotic</td>
<td>Individual antibiotics / group</td>
<td>Used in research institutions for quantitative estimation, rarely in big dairies</td>
</tr>
<tr>
<td>Snap Test</td>
<td>Receptor binding assay</td>
<td>IDEXX</td>
<td>10 min</td>
<td>Rs. 300 per test</td>
<td>Family specific</td>
<td>Not in use</td>
</tr>
</tbody>
</table>
Impact of Antimicrobial Resistance

- **700,000**
  Annual deaths currently

- **10 million**
  Projected deaths in 2050

- **$100 trillion**
  Loss to global economy till 2050

- **3.5%**
  Reduction in global GDP in 2050

**AMR is now**

- Economic problem
- Food security risk
- Development Issue
- Political challenge

- UNGA, WHO, FAO, OIE, G8, G20, G77, ASEAN, OPEC, EU

- Implementation of National Action Plan on AMR

*(Jim O’Neill Report)*
ANTIMICROBIAL RESISTANCE- A THREAT

The Bad Bugs ……

Extended spectrum β-lactamase producers

Colistin resistant Enterobacteriaceae

AmpC type β-lactamase producers

Fluroquinolone resistant Enterobacteriaceae

Vancomycin resistant and intermediate *S. aureus*

Mettalo-β-lactamase producing *Enterobacteriaceae*

Methicillin resistant *S. aureus*
Factors leading to antimicrobial resistance (AMR) in dairy animals

- Unrestricted drugs availability
- Unchecked AMU/ dosage
- Improper diagnosis
  - Selective pressure
  - Gene exchange and transmission
- Poor infection prevention & control measures
- Lack of surveillance & monitoring

Emergence of AMR strains in animals

Sharma et al, 2018
WHO PRIORITY LIST OF PATHOGENS

Priority 1: CRITICAL
- Carbapenem-resistant *Acinetobacter baumannii*
- Carbapenem-resistant *Pseudomonas aeruginosa*,
- 3rd generation cephalosporin-resistant, ESBL-producing carbapenem-resistant *Enterobacteriaceae*

Priority 2: HIGH
- *Enterococcus faecium*
- Vancomycin-resistant *Staphylococcus aureus*, Vancomycin resistant *Helicobacter pylori*
- Fluoroquinolone-resistant *Neisseria gonorrhoeae*

Priority 3: MEDIUM
- *Streptococcus pneumoniae*
- Penicillin-non-susceptible *Haemophilus influenzae*
- Ampicillin-resistant *Shigella* spp.

Diagnostic for Mastitis, Antimicrobial Residues and Resistant bacteria in dairy settings
AMR – where to look and what to look?

Indicators

- *E. coli*
- *Staph*
- *Enterococcus*
- *Pseudomonas*
- *Salmonella*
- *Klebsiella*
- *Campylobacter*

The list is not comprehensive.
Proposed draft guidelines on integrated surveillance of antimicrobial resistance

CODEX ALIMENTARIUS COMMISSION

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Agenda Item 5

CX/AMR 17/5/6
September 2017

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

AD HOC CODEX INTERGOVERNMENTAL TASK FORCE ON ANTIMICROBIAL RESISTANCE

Fifth Session

PROPOSED DRAFT GUIDELINES ON INTEGRATED SURVEILLANCE OF ANTIMICROBIAL RESISTANCE

Prepared by the Electronic Working Group led by the Netherlands and co-chaired by Chile, China and New Zealand

Codex members and Observers wishing to submit comments at Step 3 on the proposed draft Guidelines (Appendix I to this document) should do so as instructed in CL 2017/82-AMR available on the Codex webpage/Circular Letters 2017:


9/24/2018
Diagnostic for Mastitis, Antimicrobial Residues and Resistant bacteria in dairy settings
10.6 Target microorganisms and resistance determinants

Bacterial species should be chosen considering public health aspects, including the epidemiology of foodborne diseases, and should include both foodborne pathogens and indicator organisms of commensal bacteria.

*Salmonella* is a key foodborne pathogen and should therefore be included in an integrated monitoring and surveillance programme. Other foodborne pathogens like *Campylobacter* should also be strongly considered, as well as other pathogens depending on national or regional situation and risks (e.g. *Staphylococcus*, *Clostridium* or *Vibrio*).

Indicator organisms of commensal intestinal bacteria may contaminate food and can harbour transferable resistance genes. Commensal *E. coli* and *Enterococcus* spp should be used as indicators of Gram negative and Gram positive intestinal flora.

Whenever possible the monitoring and surveillance programme should include genetic and/or phenotypic analysis of particular isolates that may be a public health concern such as ESBL- AmpC and carbapenemase-producing strains.

Tests for virulence factors, AMR genes, gene transferability and gene sequencing can also be applied.

http://www.fao.org/fao-who-codexalimentarius/sh-roxy/it/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-804-05%252FWD%252Fam05_06e.pdf
Carbapenemases are now seen as a new and potentially emerging problem in food-producing animals.

All isolates of *Salmonella* spp. and *E. coli* should be screened for Carbapenem resistance.

Isolates which are resistant to 3rd or 4th generation cephalosporins should be subjected to phenotypic testing and characterization of the carbapenemase genes.

Carbapenem resistance in dairy cattle and raw milk samples should also be addressed because of specific use of cephalosporins in dairy cattle, and to the risk posed by potential consumption of raw milk.
AMR surveillance in India

➢ WHO Global Antimicrobial Resistance Surveillance System (GLASS)
➢ MoHFW /NCDC: AMR surveillance network (10 labs)
➢ ICMR: AMR Surveillance Network (4 institutions/6 labs)
➢ INFAAR: Indian Network for Fishery and Animals Antimicrobial Resistance (13 institutions)
# Prevalence of Antibiotic resistant bacteria in animals Indian scenario

<table>
<thead>
<tr>
<th>PLACE</th>
<th>SAMPLE TYPE</th>
<th>ISOLATES</th>
<th>RESISTANCE</th>
<th>RESISTANCE MECHANISM</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>West Bengal</strong></td>
<td>Mastitis milk</td>
<td>8 (E. coli)</td>
<td>5</td>
<td>Positive Horizontal transmission from human or environmental sources</td>
<td>Ghatak et al., 2013</td>
</tr>
<tr>
<td></td>
<td>Bovine mastitis</td>
<td>7 (E. coli)</td>
<td>3</td>
<td>-</td>
<td>Bandyopadhyay et al., 2015</td>
</tr>
<tr>
<td></td>
<td>Mastitis milk</td>
<td>50 gram negative</td>
<td>24</td>
<td>Extensive use of β-lactam antibiotics</td>
<td>Das et al., 2017</td>
</tr>
<tr>
<td><strong>Odisha</strong></td>
<td>Poultry (fecal)</td>
<td>252 (E. coli)</td>
<td>16</td>
<td>Indiscriminate antibiotics use</td>
<td>Kar et al., 2014</td>
</tr>
<tr>
<td></td>
<td>Cattle (milk)</td>
<td>64 (E. coli)</td>
<td>2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Hyderabad</strong></td>
<td>Meat, Egg, Raw milk</td>
<td>22 (E. coli)</td>
<td>6</td>
<td>Widespread use of antibiotics</td>
<td>Rashmeed et al., 2014</td>
</tr>
<tr>
<td><strong>Kolkata</strong></td>
<td>Mastitis milk</td>
<td>291 (Klebsiella)</td>
<td>23</td>
<td>Cross transmission between the human and animal</td>
<td>Koovapra et al., 2016</td>
</tr>
<tr>
<td><strong>Haryana (NDRI)</strong></td>
<td>Raw milk</td>
<td>139 (E. coli)</td>
<td>11</td>
<td>Transmission and acquisition of antibiotics resistance gene by plasmid and mobile genetic element</td>
<td>Amarjeet et al., 2018</td>
</tr>
</tbody>
</table>
**Method / name of test** | **Based on** | **Reference** | **Time** | **Cost** | **Application**
--- | --- | --- | --- | --- | ---
**Determinations of Susceptibility / resistance**

- Disk diffusion assay
  - Growth based with pure isolates
  - Reference method (as per CLSI / EFSA guidelines)
  - Overnight
  - ~ Rs. 500-1500 / test
  - R&D institutes / referral centers / regulatory agency / veterinary hospitals

- Dilution test

- E test

- Automated AST systems
  - Conventional / microdilution / automated plates
  - Vitek System, Micronaut Phoenix
  - 4-12 hrs
  - Rs. 2000-3000 / test
  - R&D institutions and laboratories only

**Detection of metabolic activity**

- Carba NP test
  - Colour from red to yellow
  - Due to pH shift
  - Growth based enzyme assay with pure isolates
  - 30-60 min
  - Rs. 250 per test
  - Different settings of dairy food chain / dairy farms / veterinary hospitals / milk reception docks etc.

- Nitrate reductase analysis; Resazurin microplate assay
  - Color change of growth medium due to pH change due to bacterial growth

**Genotypic Methods**

- PCR; Microarray and other Modifications
  - Different Biotechnological companies
  - 3-4 hours
  - 5000-10000 per sample
  - Clinically / research laboratories / not suitable for different setting in dairy supply chain
Food Supply chain

From farm

Agricultural production

Sale of commodities / raw material local, national international

Manufacturing packaging of food products

Sale of food products import wholesale retail trade

Consumer

Factors linked with farm to fork

❖ Un-organized milk production system
❖ In sufficient awareness about clean milk production, hazards and risks involved (HACCP system)
❖ Inadequate power supply/ or cold chain
❖ Poor linkage between milk producers and dairy processers because of active role of middle man
❖ No regulatory intervention during milk production and reception of milk at dairy units
❖ Lack of rapid field levels kits for monitoring of different microbial contaminants in milk for conducting risk assessments work and regulatory compliance are lacking
❖ Manual handling leading to post pasteurization contamination
❖ Level of automation during processing

Development of Sector wide food safety guidelines (GAP/GDP/GHP/GMP) during entire dairy supply chain involving all stake holders for regulatory compliance
The Government established the Food Safety and Standards Authority of India (FSSAI) under the Food Safety and Standards Act, 2006 (FSSA) with the mandate to lay down science based standards for food products and to regulate their manufacture, storage, distribution, sale and import, to ensure availability of safe and wholesome food.

**Main features of Integrated food law**
- Harmonization with international standards such as CODEX
- Shift from a regulatory regime to self compliance
- To lay down scientific standards and ensure availability of safe food
- Single reference point for all issues related to food safety and standards
- Clear procedures for food recall

**Key Microbial Food safety Challenges**
- Awareness creation
- Capacity building
- Infrastructure creation
- Building Research & Development Capacity
- Certification of Raw Material
- Traceability system
Awareness Creation

Poor general awareness towards the hazards associated with unsafe food practices and the best practices to be followed

Effective awareness creation programs need to be carried out by the Governmental agencies for smooth transition from the current food safety laws to the proposed system, specifically by keeping the small and medium enterprises abreast of the salient features of the law and practical issues that are likely to be faced by the manufacturers and their solutions

Capacity building programme for enhanced food safety at different stages in dairy supply chain (FBO/ regulators)
One of the critical links in the successful implementation of FSSAI is food testing laboratories.

Under the new law the manufacturers need to get their products tested every month and keep a certificate.

Hence, building up a sufficient number of accredited laboratories is of paramount importance.

Establishment of referral centre / BSL-2 laboratory for food safety monitoring / FSSAI standard compliance
Insufficient technical expertise and skilled manpower for implementation of legislation at the grass root level

- Massive efforts are required for capacity building in order to successfully implement the proposed FSSAI at the grass root level
- Well evolved training programs need to be conducted for the state, district and block level enforcement agencies
- The programs would have to equip the implementing officers with knowledge on international standards of food safety and quality thus enabling regulators to make judicious decisions relating to food contamination

Microbiological food safety challenges in the Indian dairy industry

10/10/2018
Certification of Raw Material

➢ One of the major sources of contamination in food systems occurs during the primary production stage - which is kept out of the ambit of the FSSAI

➢ Successful and holistic implementation of Food safety system would require an extensive campaign that encourages implementation of Good Agricultural Practices (GAP) at the farm level

Exclusion of primary producers from the purview of the law thus putting the onus of preventing food hazards on the manufacturers or processors
Organized manufacturers should be encouraged to take pro-active steps to ensure that GAP is adhered to by their suppliers, and a traceability system including geographic application is placed at the back-end thus reducing the risk of food contamination.
Concluding remarks

✓ Generation of scientific data on hygiene/ safety indicators in milk and milk products through National surveillance network project.
✓ Establishment of codex cells for development of food safety standards based on risk analysis
✓ Development of food safety guidelines (GAP/GDP/GHP/GMP) during entire dairy supply chain involving all stake holders
✓ Compulsory implementation of QMS and HACCP for dairy industry to ensure domestic as well as export standard compliance
✓ Capacity building programme for enhanced food safety at different stages in dairy supply chain (FBO/ regulators)
✓ Establishment of referral center /BSL-2 laboratory for food safety monitoring / FSSAI standard compliance
✓ Stronger interaction between industry and R & D institutions for enhanced innovation and know how transfer leading to better productivity in dairy sector
✓ Adoption of new technologies developed by R & D institutions by FSSAI for their regulatory compliance
✓ Development of rapid diagnostics for addressing the issues of residues, mastitis, AMR and dairy pathogen detection in dairy food chain
Diagnostic for Mastitis, Antimicrobial Residues and Resistant bacteria in dairy settings