Foodborne Outbreak Investigation and Whole Genome Sequences Analysis in Korea MFDS

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1. Foodborne Outbreak Trend in Korea
Foodborne Outbreak Trend

Foodborne outbreak increases and decreases repeatedly every year. In particular, foodborne outbreak in schools significantly changes the total number of foodborne outbreak cases.
Trend by Causative pathogens

- No. of cases: The number of Norovirus cases is the highest.
- No. of patients: The number of pathogenic *E. coli* patients is the highest.

![Bar chart showing the number of cases and patients by causative pathogens for the years 2013-2017.](chart)

- **No. of cases**
  - Pathogenic *E. coli*
  - *Salmonella*
  - *Campylobacter jejuni*
  - *C. perfringens*
  - Norovirus
  - Protozoa

- **No. of patients**
  - Pathogenic *E. coli*
  - *Salmonella*
  - *Campylobacter jejuni*
  - *C. perfringens*
  - Norovirus
  - Protozoa

*5-Year Average (2013-2017)*
The relative trend of Causative pathogens

- The relative trend of occurrence shows that *Vibrio parahaemolyticus*, pathogenic *E.coli* and *Salmonella spp.* have an increasing trend.
- *Norovirus* continues to occur.

![Graph showing the relative trend in the number of patients for different causative pathogens from 2006 to 2017.](image)
Occurrence Pattern by Season and Causative Pathogen

**Constant pattern shown by season and causative pathogens**

* C. perfringens (spring, autumn), Campylobacter, pathogenic E.coli, Vibrio parahaemolyticus (summer), Norovirus (winter)

<table>
<thead>
<tr>
<th>Major causative bacteria</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic E.coli</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norovirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. perfringens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Foodborne Outbreak Occurrence Pattern (by place)

- (No. of cases) Restaurants (62%) and (No. of patients) schools (43%) with many number of people who take school meal at a time.
2. Foodborne outbreak Investigation and Establishment of DB for Characteristics Analysis
Structure of Foodborne Outbreak Investigation in KOREA

MFDS (Headquarters)
- Coordinate the investigation of causes of food poisoning and conduct tracking.
- Participate in tracking of causes if the food poisoning spreads nationwide

Regional MFDS Office
- Track causative food
- Support mobile fast test room

Cooperate with schools with more than 50 people

City (Municipality)
- Support the investigation on causes of food poisoning (people, equipment)

Community health center
- Investigate patient cases and epidemiology
- Take sample from people

Food hygiene department
- Investigate facilities
- Investigate food, environment
- Report the result of investigation
- Take administrative measures

Voluntary reporting
- Patient, etc.

Mandatory reporting
- Doctor, oriental medicine doctor
- Those who installed and operated group meal facilities

Relevant organizations
- Blue House
- Office for Government Policy Coordination
- Ministry of Education
- Ministry of Justice
- Ministry of National Defense
- Ministry of Agriculture, Food and Rural Affairs
- Ministry of Oceans and Fisheries
- Ministry of Health and Welfare
- Ministry of Environment
- National Police Agency
- KCDC

Local Health and Environment Research Institute
Process of Foodborne Outbreak Investigation

1. Identify foodborne bacteria
2. Separation from human samples
3. Identify foodborne pathogen
4. Sample from patients (feces, etc.)
5. Separation from food
6. Identify foodborne pathogen
7. Match between patient/food separated strains
   - Homology comparison (PFGE, etc.)
8. Identify food that causes food poisoning
9. Causative food
10. Suspicious food that may cause foodborne outbreak (preservative food, etc.)
11. Food A
12. Food B
Need for Characteristics Analysis and DB for Foodborne Pathogen

- Outbreak of large scale food poisoning
  - Sample from patients
  - Separation of bacteria from human samples
  - Identify foodborne pathogen

- Suspicious food that may foodborne outbreak (preservative food, etc.)
  - Separation of bacteria from food
  - Identify foodborne pathogen

- DB system
  - Comparative analysis
    - Whether the similar types of bacteria appeared in the past or not.
    - Regions where the strains frequently appear in the past.
    - Food where the strains are separated frequently

- Track the source of contamination

Need for DB on foodborne pathogen which can be used for tracking sources of contamination with foodborne pathogen
Foodborne pathogen Resources Center

- Medium preparation
- Pathogen isolation and culture
- Freeze drying
- Analysis of characteristics
- Metagenomic analysis
- WGS analysis
- Pathogen storage

KCCF
3. Foodborne Outbreak Investigation through WGS Analysis
Establishment of Genome DB

- Establishment of genome DB: identify and analyze genome-level information on foodborne pathogens that are found in domestic food.

Data QC

Data analysis

DB accumulation

Web service

Standard whole genome
Transcriptome
Metagenome
Whole genome

0 200 400 600 800 1000 1200 1400 1600 1800 2000

2014 2015 2016 2017 2018

Standard whole genome
100 cases

Transcript
36 cases

Metagenome
756 cases

Whole genome
1168 cases

Table: Types of Genomes and Cases

<table>
<thead>
<tr>
<th>Year</th>
<th>Standard whole genome</th>
<th>Transcriptome</th>
<th>Metagenome</th>
<th>Whole genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2015</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2016</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2017</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2018</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Graph: 2018 to 2014

- Standard whole genome: 100 cases
- Transcriptome: 36 cases
- Metagenome: 756 cases
- Whole genome: 1168 cases
**Genome Homology Analysis - *Salmonella***

**SNP-based genome homology analysis results** for 54 strains separated from 5 foodborne outbreak cases caused by *Salmonella* between 2014 and 2018.

**Table. No. of SNPs per case**

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>≤14</td>
<td>&gt; 30982</td>
<td>&gt; 31892</td>
<td>&gt; 29989</td>
<td>&gt; 29991</td>
</tr>
<tr>
<td>2016</td>
<td>&gt; 30982</td>
<td>≤20</td>
<td>&gt; 31591</td>
<td>&gt; 30021</td>
<td>&gt; 30021</td>
</tr>
<tr>
<td>2014</td>
<td>&gt; 31892</td>
<td>&gt; 31591</td>
<td>≤16</td>
<td>&gt; 31571</td>
<td>&gt; 31573</td>
</tr>
<tr>
<td>2014</td>
<td>&gt; 29989</td>
<td>&gt; 30021</td>
<td>&gt; 31571</td>
<td>≤17</td>
<td>43~56</td>
</tr>
<tr>
<td>2018</td>
<td>&gt; 29991</td>
<td>&gt; 30021</td>
<td>&gt; 31573</td>
<td>≤17</td>
<td>≤17</td>
</tr>
</tbody>
</table>

**SNP analysis on 5 *Salmonella* foodborne outbreak cases confirmed that there are differences of SNP≤20 within an accident and SNP≥43 between accidents.**
SNP-based genome homology analysis results for 52 strains separated from 5 foodborne outbreak cases caused by Pathogenic *E. coli* between 2013 and 2017.

Table. No. of SNPs per case

<table>
<thead>
<tr>
<th></th>
<th>ETEC</th>
<th>EPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014 Jeonnam</td>
<td>≤11</td>
<td>&gt;20302</td>
</tr>
<tr>
<td>2013 Incheon</td>
<td>&gt;203</td>
<td>≤15</td>
</tr>
<tr>
<td>2015 Armed Forces Medical Research Institute</td>
<td>&gt;16086</td>
<td>&gt;20404</td>
</tr>
<tr>
<td>2017 Chungbuk</td>
<td>≤16086</td>
<td>&gt;8687</td>
</tr>
</tbody>
</table>

SNP analysis on 4 pathogenic *E.coli* foodborne outbreak cases confirmed that there are differences of SNP≤15 within an accident and SNP≥8687 between accidents.
Genome Homology Analysis - *Listeria*

SNP-based genome homology analysis results related to 4 strains separated from *Listeria monocytogenes* foodborne outbreak cases and 16 strains separated from monitoring and standard specification test in 2018.

Table. No. of SNPs per case

<table>
<thead>
<tr>
<th>Food poisoning that occurred in 2018 SNP≤3</th>
<th>Monitoring 1</th>
<th>Monitoring 2</th>
<th>Monitoring 3</th>
<th>Monitoring 4</th>
<th>Monitoring 5</th>
<th>Monitoring 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤3 &gt; 865 &gt; 3973 &gt; 4315 &gt; 4323 &gt; 52624 &gt; 51826</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 865 ≤2 &gt; 3986 &gt; 4305 &gt; 4343 &gt; 52650 &gt; 51840</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 3973 &gt; 3986 ≤10 &gt; 4087 &gt; 4266 &gt; 52558 &gt; 51740</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 4315 &gt; 4305 &gt; 4087 ≤17 &gt; 4311 &gt; 52635 &gt; 51808</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 4323 &gt; 4343 &gt; 4272 &gt; 4311 ≤13 &gt; 52514 &gt; 51711</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 52624 &gt; 52650 &gt; 52558 &gt; 52635 &gt; 52514 ≤15 &gt; 10673</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 51826 &gt; 51840 &gt; 51740 ≤3 &gt; 51808 &gt; 51711 &gt; 10673 ≤3</td>
<td></td>
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</tr>
</tbody>
</table>

SNP analysis results confirmed that there are differences of SNP≤3 within an accident and SNP≥865 between foodborne outbreak cases and other strains.
### Summary of Genome Homology Analysis Results

<table>
<thead>
<tr>
<th>Type</th>
<th>Food Poisoning</th>
<th>No. of SNPs in an accident</th>
<th>No. of SNPs in between accidents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella A</td>
<td>2016 Gyeongnam</td>
<td>≤14</td>
<td>29989~31892</td>
</tr>
<tr>
<td>Salmonella B</td>
<td>2016 Gyeongbuk</td>
<td>≤20</td>
<td>30021~31591</td>
</tr>
<tr>
<td>Salmonella C</td>
<td>2014 Gangwon</td>
<td>≤16</td>
<td>31571~31892</td>
</tr>
<tr>
<td>Salmonella D</td>
<td>2014 Jeonnam</td>
<td>≤17</td>
<td>43~31571</td>
</tr>
<tr>
<td>Salmonella E</td>
<td>2018</td>
<td>≤17</td>
<td>43~31573</td>
</tr>
<tr>
<td>Pathogenic E.coli F</td>
<td>2014 Jeonnam</td>
<td>≤11</td>
<td>20272~20404</td>
</tr>
<tr>
<td>Pathogenic E.coli G</td>
<td>2013 Incheon</td>
<td>≤15</td>
<td>8687~20302</td>
</tr>
<tr>
<td>Pathogenic E.coli H</td>
<td>2015 Armed Forces Medical Research Institute</td>
<td>≤11</td>
<td>16086~20404</td>
</tr>
<tr>
<td>Pathogenic E.coli I</td>
<td>2017 Chungbuk</td>
<td>≤8</td>
<td>8687~20272</td>
</tr>
<tr>
<td>Listeria     J</td>
<td>2018</td>
<td>≤3</td>
<td>865~52624</td>
</tr>
</tbody>
</table>

MFDS SNPing was applied to utilize homology analysis method. It is necessary to expand and validate more foodborne pathogens for SNP-based genome homology analysis.
Foodborne Pathogen Genome Network

WGS data
Standardize & Transfer
[Total genome information: 1,000 (’18) → 3,000 (’19) → 5,500 (’20) → 8,500 (’21) → 12,000 (’22)]

Isolates from outbreak
Isolates from contaminated food/environment

Foreign Genome DB

GUI-based Analysis Reports
- Identification of causes of foodborne disease
- SNPing and Clustering

Genome characterization
- Comparative genomic analysis
- Virulence marker search
- Domestic vs Foreign isolates comparison

MFDS and regional offices
Relevant organizations (National Institute of Fisheries Science, National Institute of Animal Science)
Health and Environment Research Institute
Domestic researchers
Database expansion for foodborne pathogen using metagenomic analysis pipeline

Application of metagenomic sequencing to Food Safety

Food samples

MFDS pipeline

Probiotics Database

Reference Genome (1)

Reference Genome (2)

Reference Genome (3)

Food-borne Pathogen Database

Reference Genome (a)

Reference Genome (b)

Reference Genome (c)

Probiotic labeling and quality control

Detection of food-borne pathogens
Thank you for attention

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