The use of Omics in Hazard Characterisation

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Haddad et al. submitted
Next generation Microbiological Risk Assessment
- Potential of Omics data for Hazard Characterisation -

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Microbiological Risk Assessment

- Uncertainties & variability
  food product, organisms, host = biological entities

Various food processing, sources of contamination and organisms

- Variation in the many relevant factors
- Uncertainty results from imperfect knowledge

Number of cases

figure: courtesy of J.M Membre, INRA Nantes
Hazard Characterisation

CAC, 2016: ”The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food. For chemical agents, a dose–response assessment should be performed. For biological or physical agents, a dose–response assessment should be performed if the data are obtainable.”
Hazard Characterisation

Data obtained from:
- Animal or cell culture model systems
- Outbreaks
- General Epidemiology

⇒ All containing much uncertainty and variability

⇒ Translation, effects biology (organism, food host) effects food (pH, fat), stomach, intestinal flora,
Use of NG-Omics

Use of genetic determinants
  measure for pathogenicity
  measure for virulence
  measure of severity
  dose-response

Use of transcriptomics / metabolomics
Use of genetic determinants

Some organisms are pathogenic, others are not.

Opportunistic to real pathogens: definitions!

Links between genetic determinants and pathogenicity (from “simple” to complex pathways)

→ Example: No ces-gene no cereulide!
Use of genetic determinants

<table>
<thead>
<tr>
<th>Example of sequence of a more and more stricter definition of pathogenic potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEC = (stx1 OR stx2)</td>
</tr>
<tr>
<td>STEC = (stx1 OR stx2) AND an attachment factor like genetic element</td>
</tr>
<tr>
<td>STEC = (stx1 OR stx2) AND known attachment factor</td>
</tr>
<tr>
<td>STEC = (stx1 OR stx2) AND (Eae OR (aaiC and aggR))</td>
</tr>
<tr>
<td>STEC = (stx1 OR stx2) AND (Eae)</td>
</tr>
</tbody>
</table>
Use of genetic determinants

- Traditionally viewed/regulated by serotype
  - Dutch guideline of 2014
    - for high risk ready to eat (RTE) foods
    - all STEC with (stx1 OR stx2) are considered unacceptable,
  - low risk food products (to be cooked)
  - only STEC’s that have (stx1 AND/OR stx2) AND [(eae) OR (aaiC AND aggR)] AND belonging to serotypes (O26, O103, O111, O145, O157, O104, O45, O121 en O174)
Level of virulence

**Sequence data:** Genetic data (SNPs) + epidemiological and phenotypic analysis (*in vitro* attachment to epithelial cells as a proxy for virulence)

**Transcriptomics and proteomics approaches:**
- characterisation of the physiological state of pathogens
- considering the “food chain-human gastrointestinal track continuum” to predict food processing and conservation conditions effect on pathogen’s virulence and toxin prod.
- towards biomarkers of virulence?

**Main challenges:**
(i) correlation biomarker response and illness conditions
(ii) to quantify the correlation
<table>
<thead>
<tr>
<th>Omic methods</th>
<th>Type of biomarker</th>
<th>Example (from literature)</th>
<th>Type of response</th>
<th>Reproducibility</th>
<th>Remarks and ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomics</td>
<td>Gene (CDS)</td>
<td>stx of <em>E. coli</em></td>
<td>Qualitative</td>
<td></td>
<td>Lindsey et al., 2016</td>
</tr>
<tr>
<td></td>
<td>SNP</td>
<td>stx of <em>E. coli</em></td>
<td>Qualitative</td>
<td></td>
<td>Pielaat et al., 2015</td>
</tr>
<tr>
<td></td>
<td>Multiple copies</td>
<td>Neurotoxin genes of <em>Clostridium botulinum</em></td>
<td>Qualitative</td>
<td></td>
<td>Peck and van Vliet, 2016</td>
</tr>
<tr>
<td>Transcriptomic</td>
<td>mRNA</td>
<td>SPI-1 genes or <em>hil1A</em> of <em>Salmonella enterica</em></td>
<td>Quantitative</td>
<td>2 biological replicates</td>
<td>Comparison between two different serotypes. Elhadad et al., 2016</td>
</tr>
<tr>
<td>Proteomic</td>
<td>protein</td>
<td>TypA of <em>Cronobacter sakazakii</em></td>
<td>Quantitative</td>
<td>3 technical replicates, but no biological replicate</td>
<td>Comparison between virulent and non-virulent strains. Du et al., 2015</td>
</tr>
<tr>
<td>Metabolomic</td>
<td>metabolite</td>
<td>Cereulide toxin of <em>Bacillus cereus</em></td>
<td>Quantitative</td>
<td></td>
<td>Biesta-Peters et al. 2010; Marxen et al., 2015</td>
</tr>
</tbody>
</table>
The severity of the outcome

Both much information from microorganism and host at transcriptomics and proteomics level can give insight

→ better characterization of the pathogen using omics technologies to assess:
the presence, diversity, expression of genes encoding toxins, toxin-assembling complexes, and virulence factors

→ biomarkers predicting the severity of clinical signs or relapses
⇒ for clinical & diagnostic purposes

→ towards prediction of the severity of the outcome?
Dose Response *Salmonella*

How does it move based on strain, serovar, food, host, .... ?
Can we segregate and reduce uncertainty or variability?
- Quantitative, Generic, Transparent
Regulatory network of virulence genes of *Salmonella Typhimurium*
Implications

- great developments and use in outbreak investigation and epidemiology
  + huge data and information collection
  ! standards, harmonization
- much “clinical” focussed
- QMRA: level of the dose, behaviour of the organism in the food, the state of the organism, behaviour during the disease process
- strain variation
  - more specific segregation in virulence/pathogenicity
  - validation ?
- uncertainty
Conclusions

- Next Generation Omics (NG-Omics) impacts the future of hazard characterisation

- NG-Omics improves understanding of variability and limits uncertainty in the dose-response relation

- NG-Omics improves understanding of pathogenicity, virulence and severity of disease outcomes

- Selection of virulence biomarkers using NG-Omics and modelling can help in prediction of pathogenicity and severity of the disease
Thanks