Quantification and characterization of ESBL/AmpC-producing \textit{Enterobacteriaceae} in retail seafood in Germany

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Background

The use of $\beta$-lactams for disease treatment & prevention

Increasing resistance to $\beta$-lactams in bacteria of human & animals

The production of $\beta$-lactamases
The most common mechanism of $\beta$-lactam resistance in Gram (-) bacteria (Smet et al., 2010)

The extended-spectrum $\beta$-lactamases (ESBL)

AmpC $\beta$-lactamases
ESBL/AmpC-producing *Enterobacteriaceae*

an **emerging public health concern** (failures in treatment for infections)
Objectives

- To determine the prevalence, to investigate the quantitative load, and to characterize ESBL/AmpC-producing *Enterobacteriaceae* in retail seafood in Germany
Materials and Methods

Sample collection

- **160 samples** (80 shrimp and 80 bivalves)
- Supermarkets and seafood shops in Berlin, Germany
- December 2015 to August 2016

- **Origin countries:** Bangladesh (14), Denmark (14), Ecuador (12), France (7), Germany (8), India (10), Ireland (12), Italy (17), The Netherlands (12), Spain (1), Vietnam (4), unknown (49)
Procedure for isolation and identification of ESBL/AmpC-producing Enterobacteriaceae

**Qualitative analysis**

1. 10g of sample + 90ml of LB broth
2. 37°C/24h
3. MAC-CTX agar (37°C/24h)
4. Species identification by MALDI TOF
5. Store at -80°C
   - Detect ESBL/AmpC genes by multiplex qPCR & mPCR (Nicole et al., 2014 & Perez-Perez & Hanson, 2002)
   - Sequencing of β-lactamase genes

**Quantitative analysis**

1. 10-fold dilutions
2. Spread plating on MAC-CTX agar (37°C/24h)
3. Counting
4. Disc diffusion method (CLSI, 2012 & Sabia et al., 2012)

ESBL positive
AmpC positive
Results

Prevalence of ESBL/AmpC-producing *Enterobacteriaceae* in seafood

* No significant difference between prevalence of ESBL/AmpC-producing *Enterobacteriaceae* in shrimp and bivalves (p > 0.05)
# Origin of samples

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>No. of samples</th>
<th>No. of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Denmark</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Ecuador</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>France</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Germany</td>
<td>8</td>
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</tr>
<tr>
<td>India</td>
<td>10</td>
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<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Italy</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vietnam</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Unknown</td>
<td>49</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>160</strong></td>
<td><strong>34</strong></td>
</tr>
</tbody>
</table>
Prevalence of ESBL/AmpC-producing *Enterobacteriaceae* in different seafood species

- **White leg shrimp**: 12% (Neg), 88% (Pos)
- **Black tiger shrimp**: 27.3% (Neg), 72.7% (Pos)
- **Mussels**: 20.4% (Neg), 79.6% (Pos)
- **Venus clams**: 20% (Neg), 80% (Pos)
- **Razor shells**: 0% (Neg), 100% (Pos)
- **Cockles**: 60% (Neg), 40% (Pos)

* No significant difference between prevalence of ESBL/AmpC-producing *Enterobacteriaceae* in different seafood species (p > 0.05)
Counts of ESBL/AmpC-producing *Enterobacteriaceae* in seafood (n = 160)

- Not detected: 78.7%
- Detected, < 100 CFU/g: 19.4%
- Detected, 100 – 1000 CFU/g: 1.9%
Summary of ESBL/AmpC-producing *Enterobacteriaceae* isolates (n = 45)

**ESBL/AmpC-producing *Enterobacteriaceae* isolates:**
- *Citrobacter braakii* (1)
- *Citrobacter freundii* (5)
- *Enterobacter aerogenes* (1)
- *Enterobacter asburiae* (1)
- *Enterobacter cloacae* (6)
- *Escherichia coli* (12)
- *Hafnia alvei* (3)
- *Klebsiella pneumoniae* (13)
- *Leclercia adecarboxylata* (1)
- *Morganella morganii* (1)
- *Pantoea septica* (1)
Characterization of β-lactamase genes in *Enterobacteriaceae* isolates

<table>
<thead>
<tr>
<th>β-lactamase genes</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 genes: CTX-M + SHV + DHA</td>
<td>6.7</td>
</tr>
<tr>
<td>2 genes: CTX-M + TEM, SHV + TEM, SHV + ACC, SHV + DHA</td>
<td>17.8</td>
</tr>
<tr>
<td>1 genes: CTX-M, SHV, TEM, ACC, CMY, DHA, MIR, ACT</td>
<td>62.2</td>
</tr>
<tr>
<td>Unknown genes</td>
<td>13.3</td>
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</tbody>
</table>

**Frequency**
Conclusion

- The first report of ESBL/AmpC-producing *Enterobacteriaceae* in retail seafood
- Evidence of hazard potential of seafood containing ESBL/AmpC-producing *Enterobacteriaceae*
  - Low count but high prevalence
Acknowledgment
Thank you for your attention!

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