OCCURRENCE AND ANTIBIOTIC RESISTANCE OF *LISTERIA MONOCYTOGENES* ISOLATED FROM FRESH WATER FISH IN EAST COAST MALAYSIA

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What is *Listeria monocytogenes* and why bother?
What is *L. monocytogenes*?

- Gram positive rods, non-spore forming
- Singly, arranged in Y or V forms or short chains.
- Ubiquitously found in diverse environment
- Tumbling motility at 20-25°C; less motile at 37°C
- Psychrotrophs
- Acid tolerance
- Biofilm formation
• 13 serotypes
• over 98% of isolates from human listeriosis belong to only four serotypes: 1/2a, 1/2b, 1/2c and 4b (Swaminathan and Gerner-Smidt 2007)
• More than 50% of *L. monocytogenes* recovered from foods and the environment are serotype 1/2 (especially 1/2a and 1/2b), while serotype 4b strains are the most prevalent cause of foodborne outbreaks of human listeriosis (Nho et al., 2015)
What is *L. monocytogenes*?
Transmission and clinical signs of listeriosis in animals and humans

Dhama et al.(2015). Veterinary Quarterly. Vol. 35, No. 4,
Major Outbreaks of Listeriosis Over The Last Decade

- **South Africa (2017, 2018):** Between January, 2017, and May 16, 2018, there have been 1034 laboratory-confirmed cases of listeriosis, more than 400 (42%) in neonates, and 204 deaths.

- **Australia:** January-April 2018, 20 outbreak cases of listeriosis were reported. 7 deaths and 1 miscarriage associated with the outbreak.

- **In European Union report (2013):** 1763 confirmed cases; 191 deaths; France with the highest death rate of 64.

- **In United States (2011):** Listeriosis outbreak across 28 US States; 147 confirmed cases, 33 deaths; cantaloupee (spanspek)

- **In Canada (2008):** 57 confirmed cases, 23 deaths; cold meat cuts from a Toronto Maple Leaf Food Factory.
Problem Statement

- 20-30% fatality rate;
- Economic burden (USDA, 2015)
- Antiobiotic resistance (Lee et al., 2017)
- 6th biggest seafood consumer globally (FAO, 2013)
<table>
<thead>
<tr>
<th>Research Questions</th>
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<tbody>
<tr>
<td><strong>01</strong></td>
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<td><strong>02</strong></td>
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<td><strong>03</strong></td>
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Research Objectives

01 To determine the occurrence of *L. monocytogenes* isolated from fresh water fish in East Coast Malaysia

02 To investigate the clonal diversity of isolated *L. monocytogenes*

03 To compare the antibiogram pattern of isolated *L. monocytogenes* from different serotypes
Antibiotic resistance gene detection

Sample collection n= 113

Bacterial isolation and detection (ISO-11290-1)

Biochemical tests

Molecular detection

Antimicrobial susceptibility testing (AST)

Serotyping

(Doumith et al., 2004; Ryu et al., 2013)
RESULTS AND DISCUSSION

Finding 1: 24/113 isolates were found to be presumptive positive for *Listeria* spp.

- Gram positive short rod
- Catalase positive
- Oxidase negative
- Motile

Hydrolyse esculin → black precipitate

Hydrolyse chromogenic substrate → bluish-green colony
Figure 1: Multiplex PCR using Lin0464 (749bp), Lis0333 (673bp), LisAll (463bp), PrS (370bp) & prfA (274bp) primers were run on 2.5% agarose gel at 90 V for 50 min.

Finding 2: *Listeria* Species Identification
## Finding 3: Occurrence of *Listeria* spp. in fresh water fish

<table>
<thead>
<tr>
<th>Fish species/sources</th>
<th>No. of samples</th>
<th><em>L. monocytogenes</em> n (%)</th>
<th><em>L. innocua</em> n (%)</th>
<th>Unidentified <em>Listeria</em> spp. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clarias gariepinus</strong></td>
<td>45</td>
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<td>-</td>
<td>-</td>
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<tr>
<td><strong>Channa striata</strong></td>
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<td>-</td>
<td>1 (7.7)</td>
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<td>1 (7.7)</td>
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<td><strong>Pangasianodon hypophthalmus</strong></td>
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<td>1 (6.7)</td>
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<tr>
<td><strong>Water sample</strong></td>
<td>8</td>
<td>1 (12.5)</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>Other fresh water fish</strong></td>
<td>19</td>
<td>-</td>
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<tr>
<td><strong>Total</strong></td>
<td>113</td>
<td>4 (3.5)</td>
<td>2 (1.7)</td>
<td>2 (1.7)</td>
</tr>
</tbody>
</table>
Finding 4: Serotyping of *Listeria monocytogenes*

Figure 2: Multiplex PCR using Lmo1118 (906bp), Lmo0737 (691bp), Lm_ORF2110 (597bp), Lm_ORF2819 (471bp) & PrS (370bp) primers were run on 2.5% agarose gel at 100 V for 50 min.
# Finding 5: Antimicrobial Susceptibility Testing

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- **Red**: Resistant
- **Yellow**: Intermediate
- **White**: Susceptible

*(CLSIFDA, 2013)*
Figure 3: Multiplex PCR using TetK (614bp), TetL (739bp), TetM (405bp), TetS (589bp) & Int-Tn (525bp) primers were run on 2.5% agarose gel at 90 V for 50 min.
The tetM sequence is showing 100% similarity with the sequence found in Enteroccus faecalis, MRSA, Streptococcus pneumoniae, etc.
**Summary Findings**

**L. monocytogenes**
- 3 isolates: 1/2b strain
- 1 isolate: 4b \(\rightarrow\) acquired tetracycline resistance

**L. innocua**
- 1 isolate \(\rightarrow\) acquired tetracycline resistance
- 1 isolate \(\rightarrow\) ampicillin & penicillin resistance

**Unidentified Listeria spp.**
- 1 isolate \(\rightarrow\) bacitracin resistance
- 1 isolate \(\rightarrow\) bacitracin resistance & streptomycin resistance
Implications

Zoonotic Diseases
spread BETWEEN animals and people

Isolation of *Listeria monocytogenes* from fresh water fish proven its destructive potential to cause foodborne zoonotic listeriosis
Implications

The presence of resistance gene in plasmid DNA highlights the potential risk of spreading that gene between different bacteria.
ONE HEALTH

Healthy People
Healthy Animals
Healthy Environment
References


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Any questions?

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