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

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WHERE ARE WE FROM?

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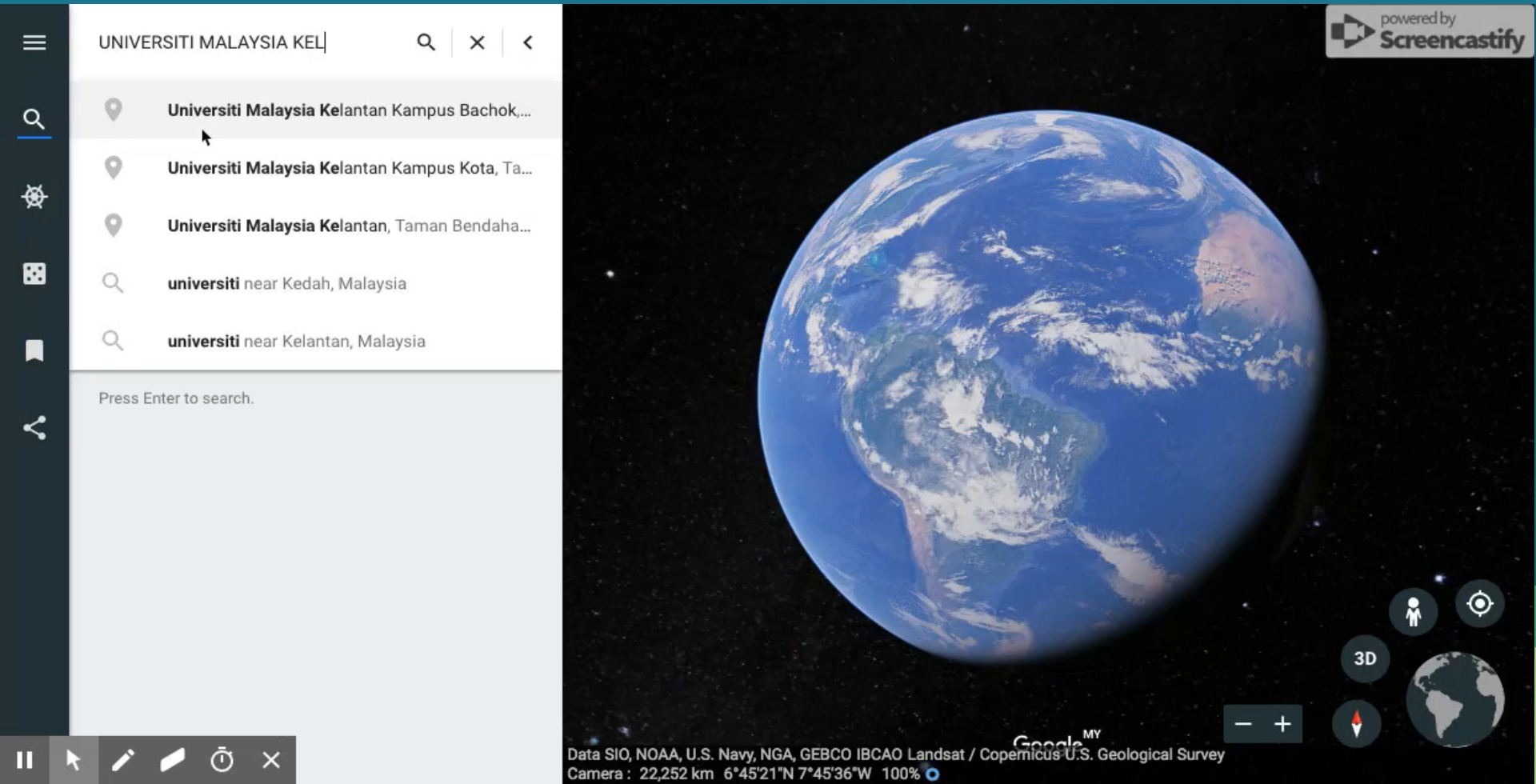
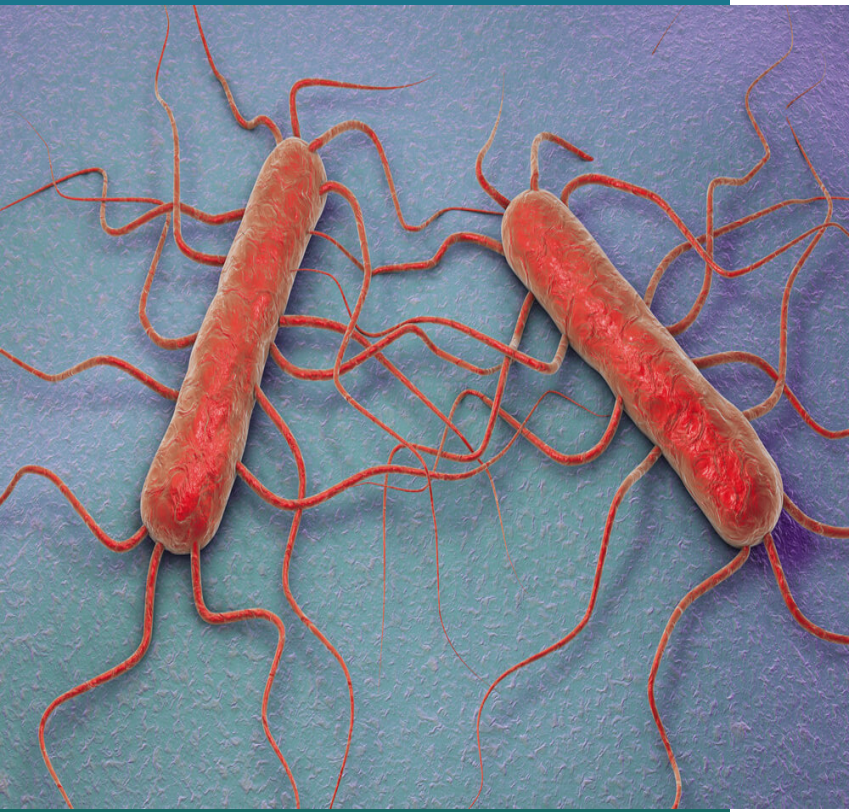
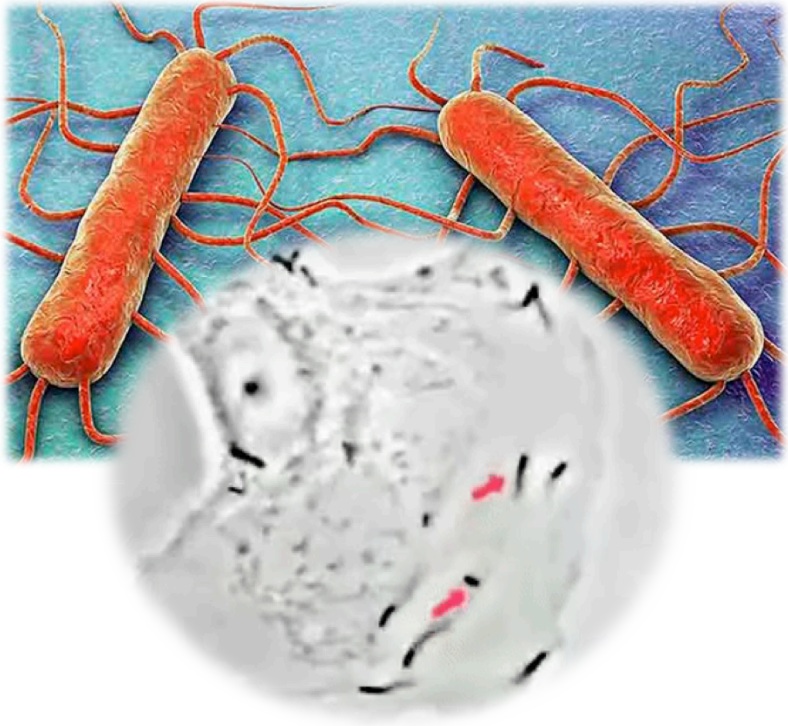




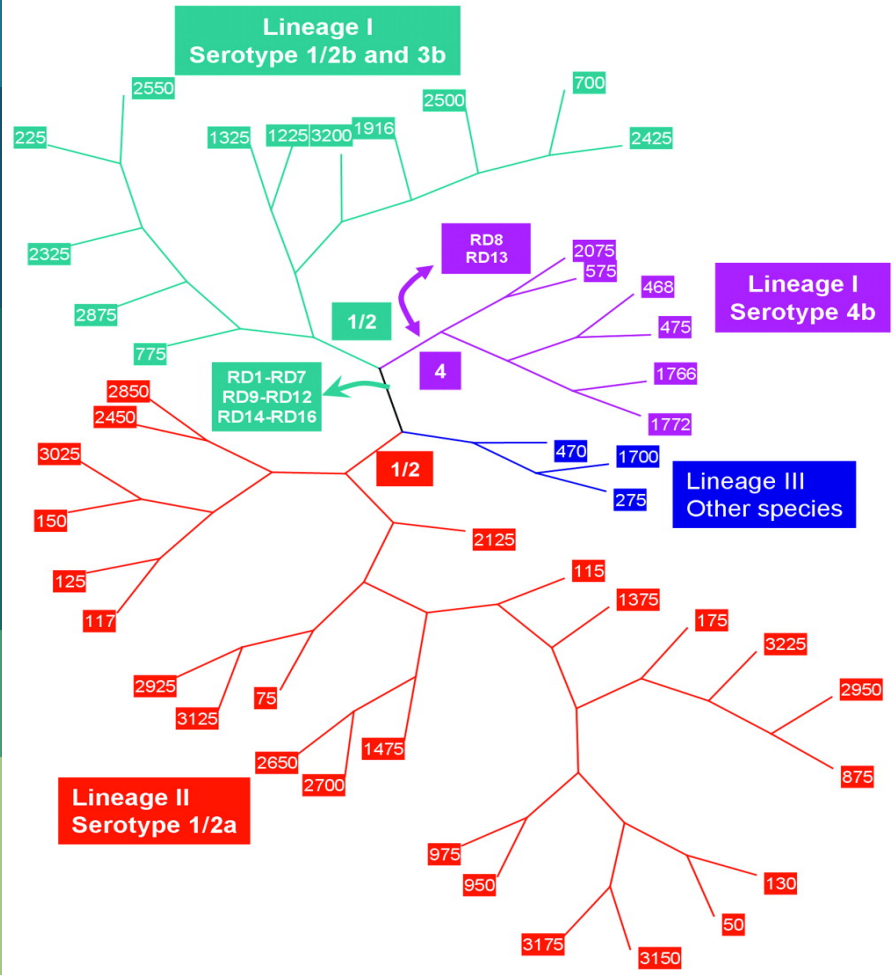
Introduction

What is *Listeria monocytogenes* and why bother?

What is *L. monocytogenes*?



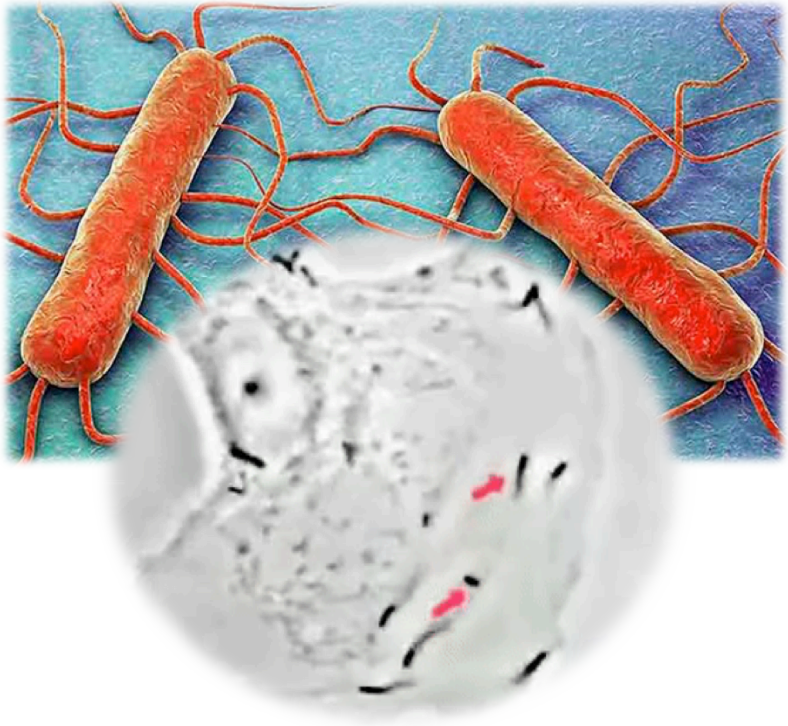
- Gram positive rods, non-spore forming
- Singly, arranged in Y or V forms or short chains.
- ubiquitously found in diverse environment
- Tumbling motility at 20-25°C; less motile at 37°C
- Psychotrophs
- Acid tolerance
- Biofilm formation

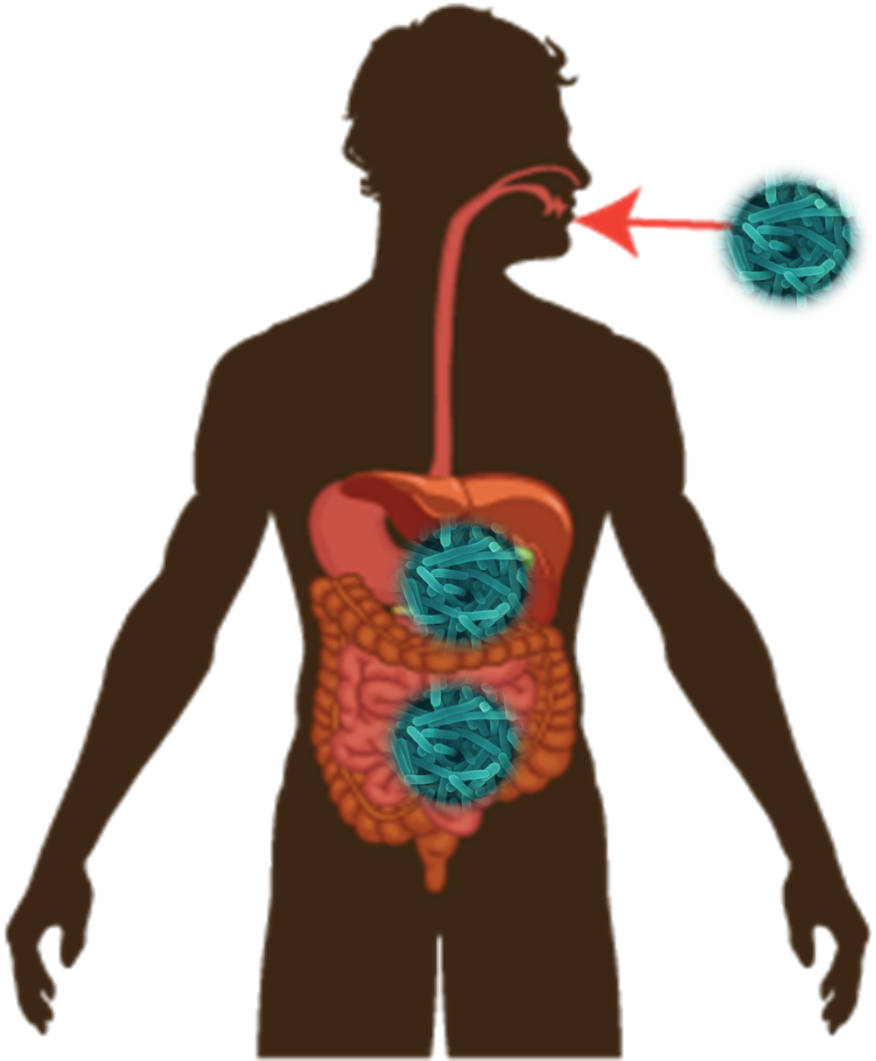


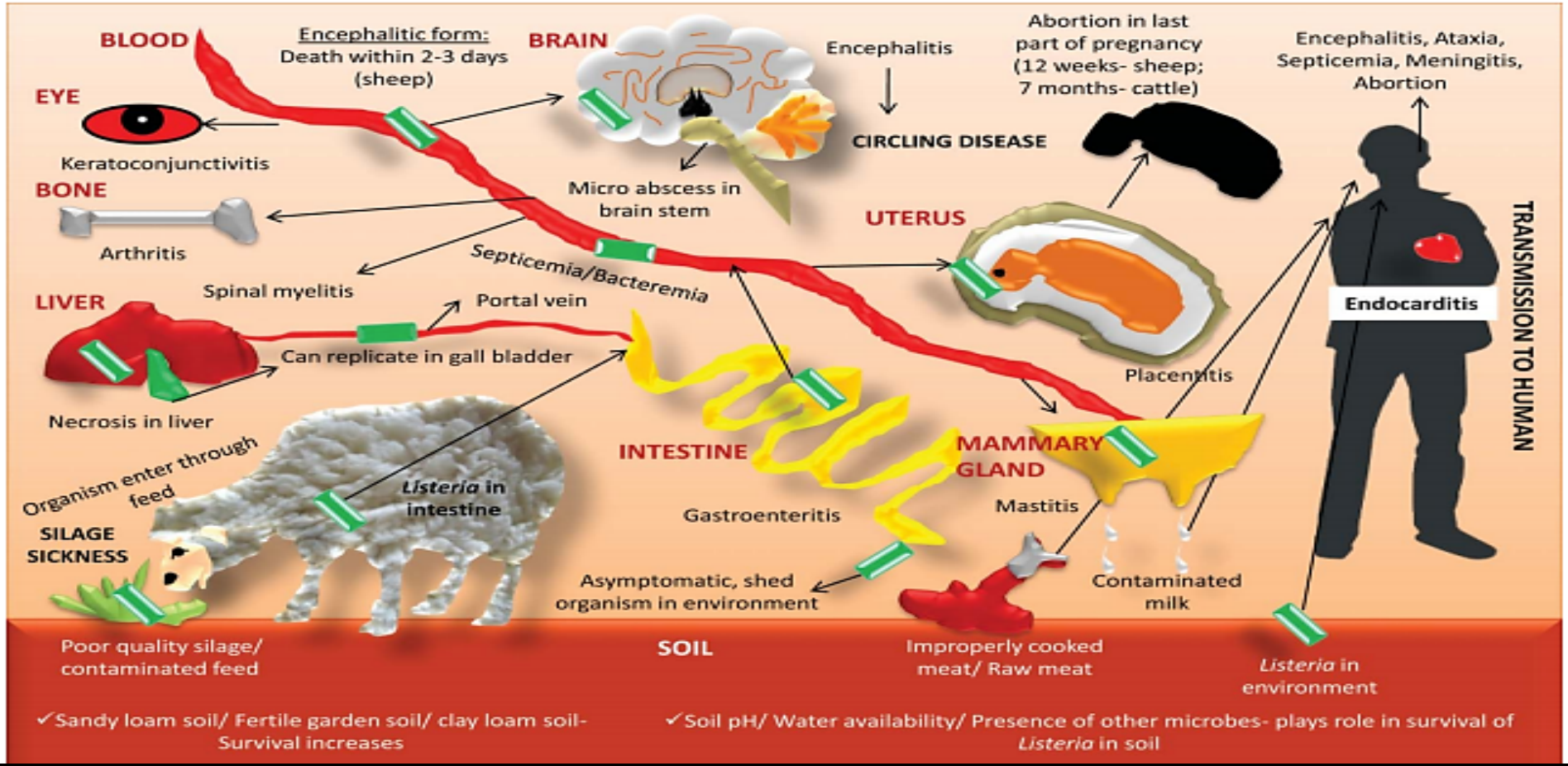
Model for evolution of the *L. monocytogenes*

- 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*?



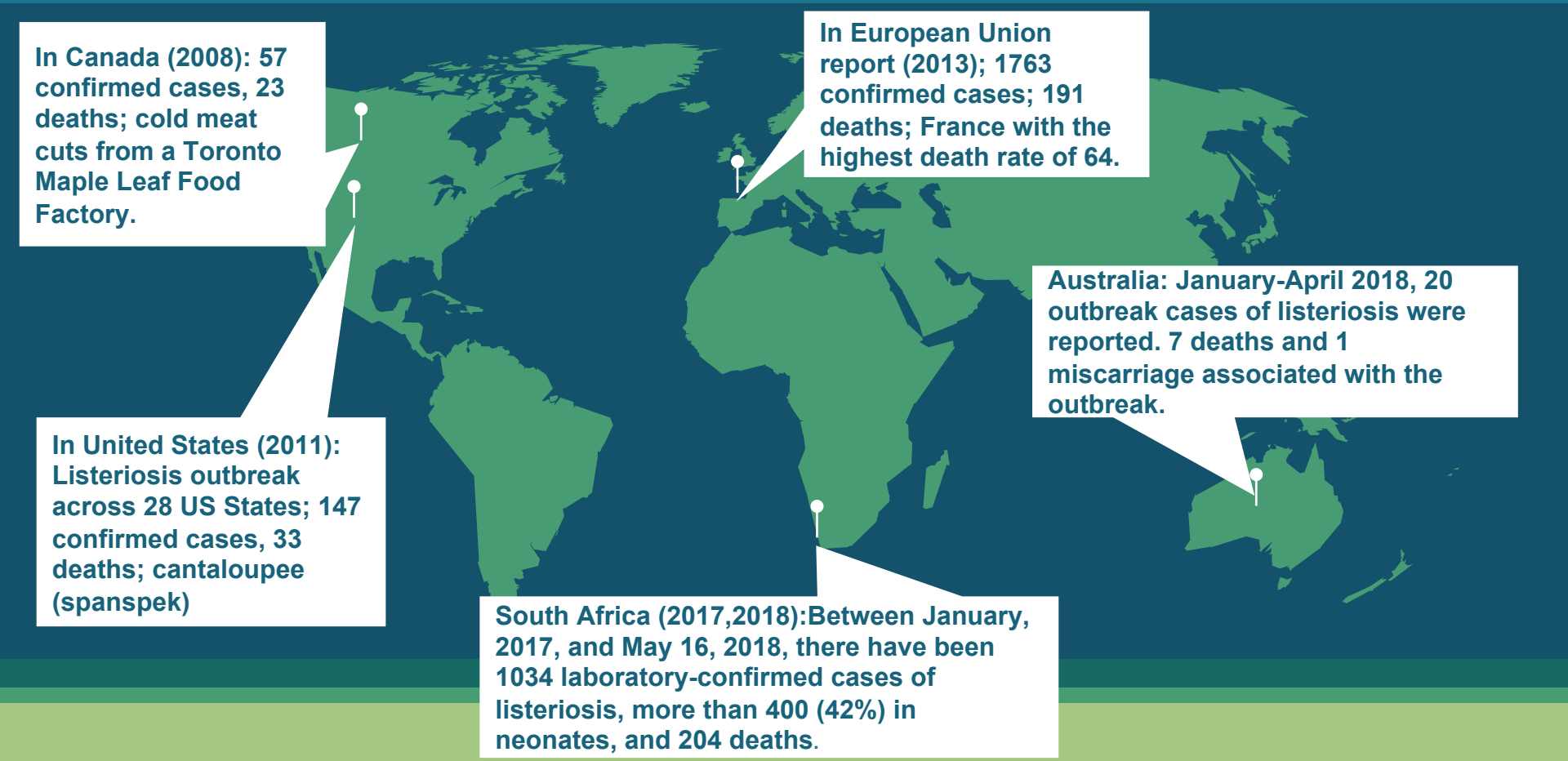




Transmission and clinical signs of listeriosis in animals and humans

Dhama et al.(2015).Veterinary Quarterly .Vol. 35, No. 4,

Major Outbreaks of Listeriosis Over The Last Decade



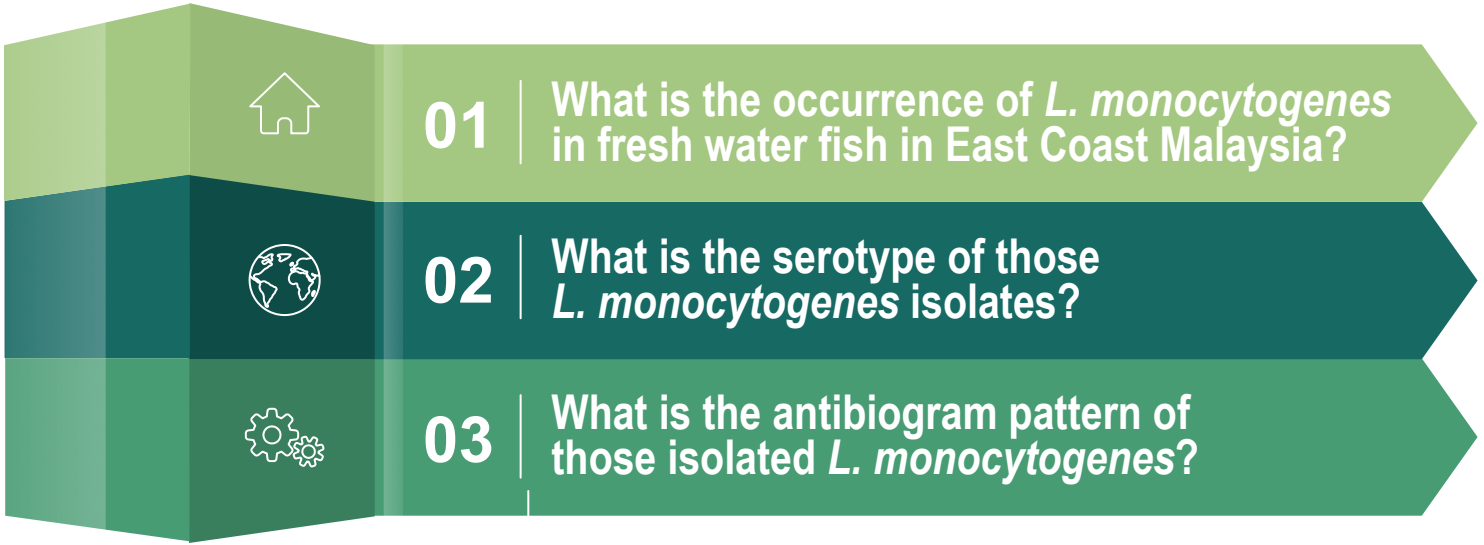
Problem Statement

20-30% fatality rate; Economic burden (USDA, 2015)

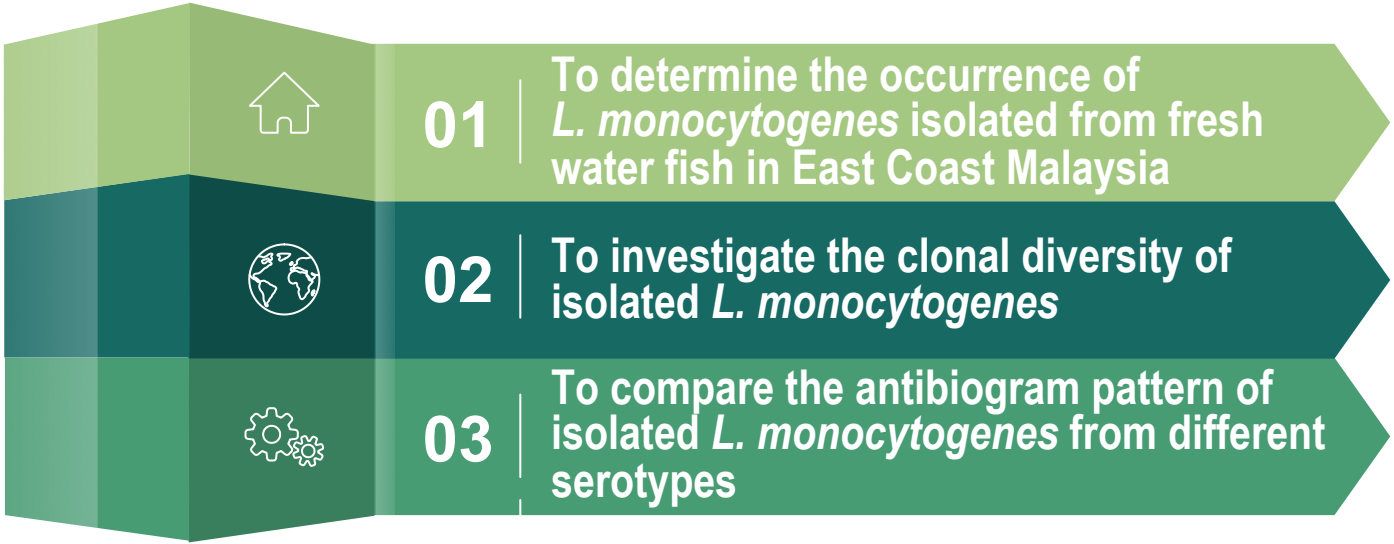
Antiobiotic resistance (Lee et al., 2017)

6th biggest seafood consumer globally (FAO, 2013)

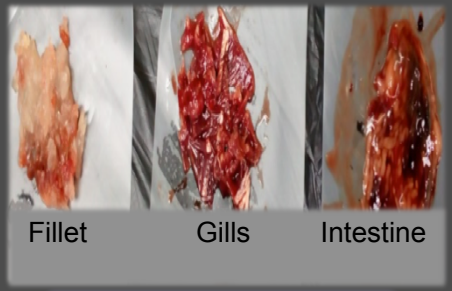
Research Questions



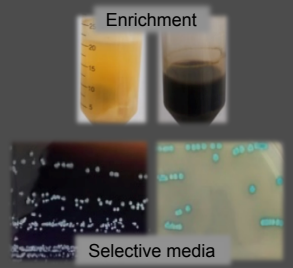
Research Objectives



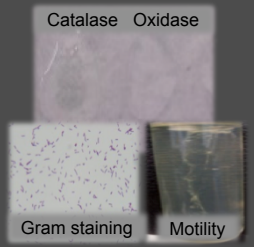
RESEARCH FLOW CHART



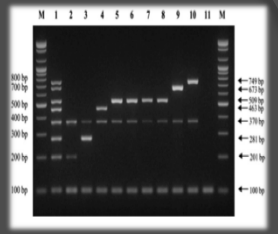
Sample collection
n= 113



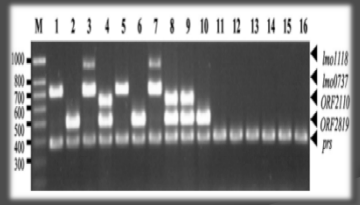
Bacterial isolation and detection (ISO-11290-1)



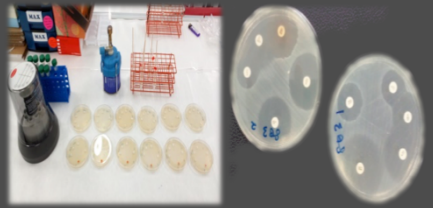
Biochemical tests



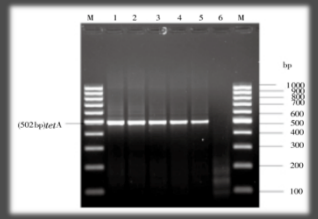
Molecular detection



Serotyping



Antimicrobial susceptibility testing (AST)

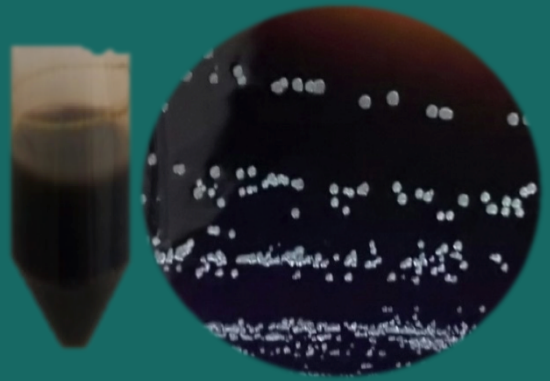


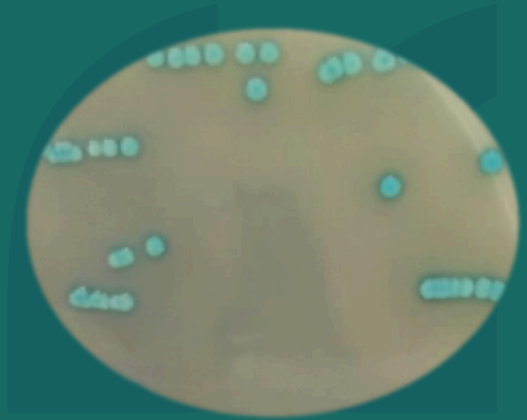
Antibiotic resistance gene detection

(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

Finding 2: *Listeria* Species Identification

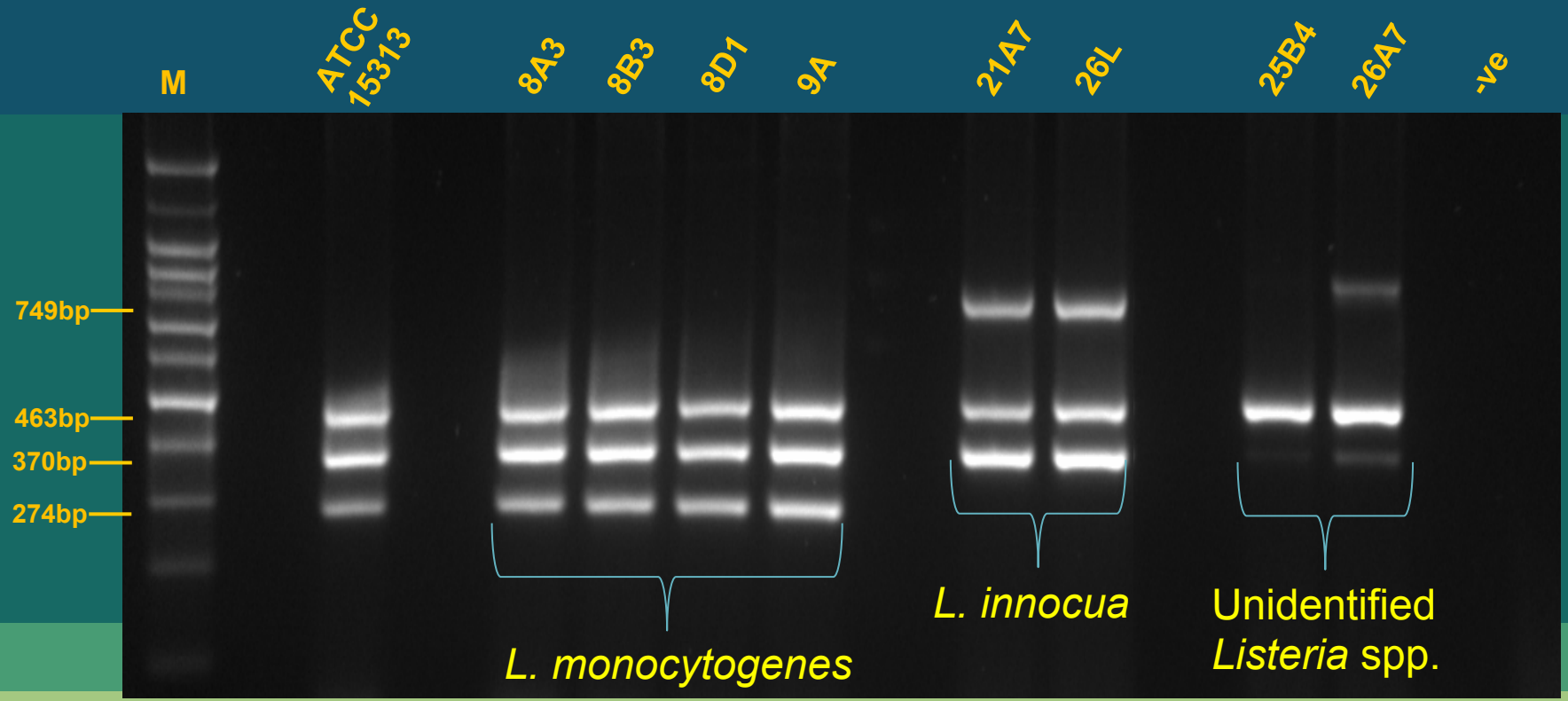


Figure 1 : Multiplex PCR using Lin0464 (749bp), Lis0333 (673bp), LisAll (463bp), PrS (370bp) & prfA (274bp) primers were run on 2.5% agarose gel at 90 V for 50 min.

Finding 3: Occurrence of *Listeria* spp. in fresh water fish

Fish species/ sources	No. of samples	*L. monocytogenes* n (%)	*L. innocua* n (%)	Unidentified Listeria spp. (%)
Clarias gariepinus	45	3 (6.7)	-	-
Channa striata	13	-	1 (7.7)	1 (7.7)
Anabas testudineus	13	-	-	1 (7.7)
Pangasianodon hypophthalmus	15	-	1 (6.7)	-
Water sample	8	1 (12.5)	-	-
Other fresh water fish	19	-	-	-
Total	113	4 (3.5)	2 (1.7)	2 (1.7)

Finding 4: Serotyping of *Listeria monocytogenes*

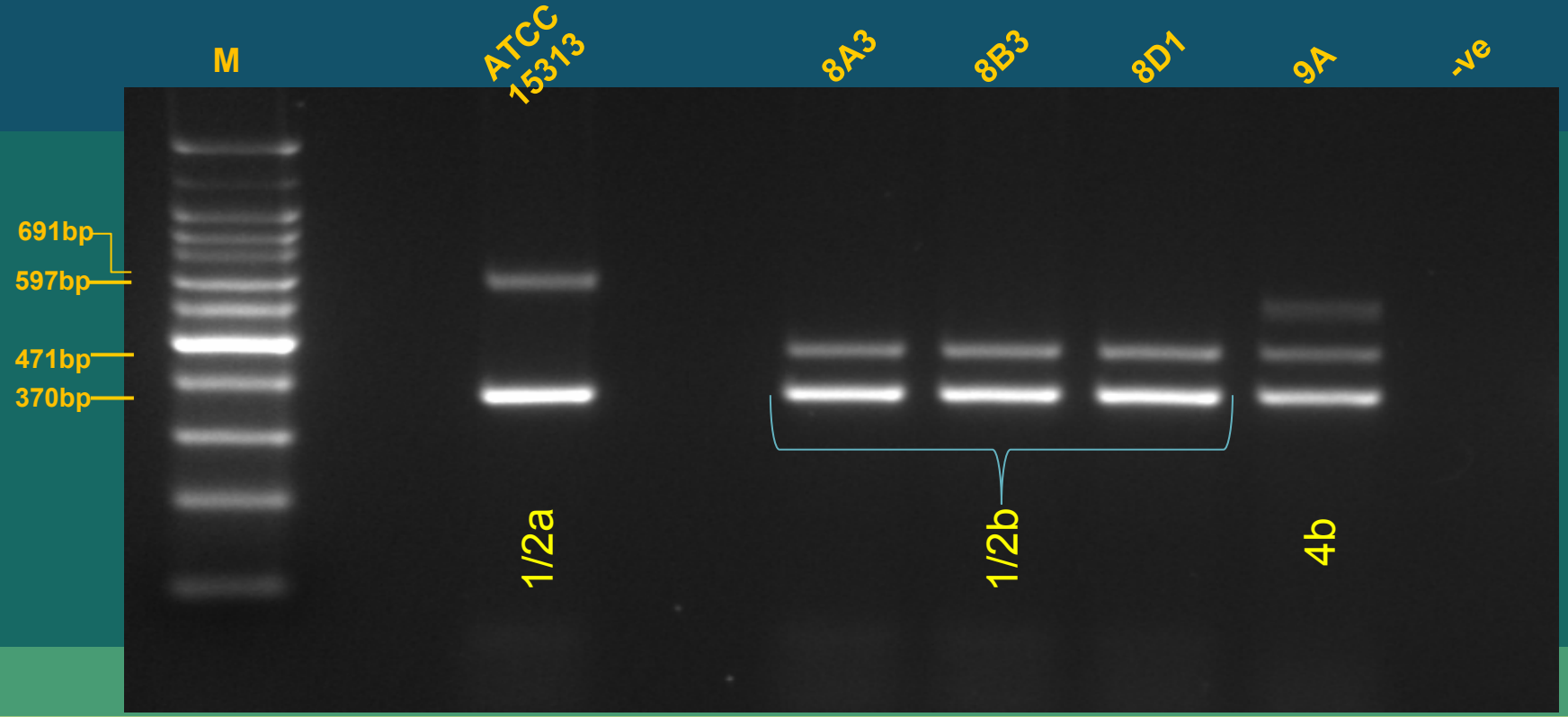


Figure 2 : Multiplex PCR using Lmo1118 (906bp), Lmo0737 (691bp), Lm_ORF2110 (597bp),Lm_ORF2819 (471bp) & PrS (370bp) primers were run on 2.5% agarose gel at 100 V for 50 min.

Finding 5: Antimicrobial Susceptibility Testing

	Zone of inhibition (mm)													
	K	VA	S	TE	P	C	E	RD	AMP	AML	B	FOX	CN	DA
ATCC	38.7	24.5	24.0	46.0	45.0	25.0	38.5	34.5	44.0	41.0	18.0	19.5	30.5	16.5
8A3	25.3	25.5	22.0	42.5	38.0	32.0	37.0	37.5	31.0	38.0	20.0	17.0	26.0	17.5
8B3	28.3	21.5	19.5	38.0	31.0	30.5	34.5	32.5	35.0	33.0	18.0	17.0	26.0	17.5
8D1	28.5	23.0	21.5	40.5	35.0	34.0	35.0	37.5	35.0	37.0	19.0	17.0	26.0	20.0
9A	28.7	19.5	17.7	11.0	30.0	29.0	31.5	30.5	33.0	30.0	18.0	15.0	23.5	14.0
21A7	27.3	21.5	18.0	13.0	33.0	28.5	30.5	30.5	30.0	32.0	16.5	14.5	22.0	12.0
26L	26.5	17.0	18.0	34.0	20.0	27.5	30.0	28.5	21.0	32.0	17.0	14.0	23.0	11.0
25B4	25.0	20.0	11.5	33.0	30.5	26.0	25.0	26.0	26.0	27.5	4.0	15.0	22.0	0.0
26A7	26.0	20.0	13.0	32.0	28.0	25.0	24.0	25.0	20.5	30.0	3.5	18.0	24.0	0.0

Resistant Intermediate Susceptible

(CLSIFDA, 2013)

Finding 6: Antibiotic Resistance Gene Detection

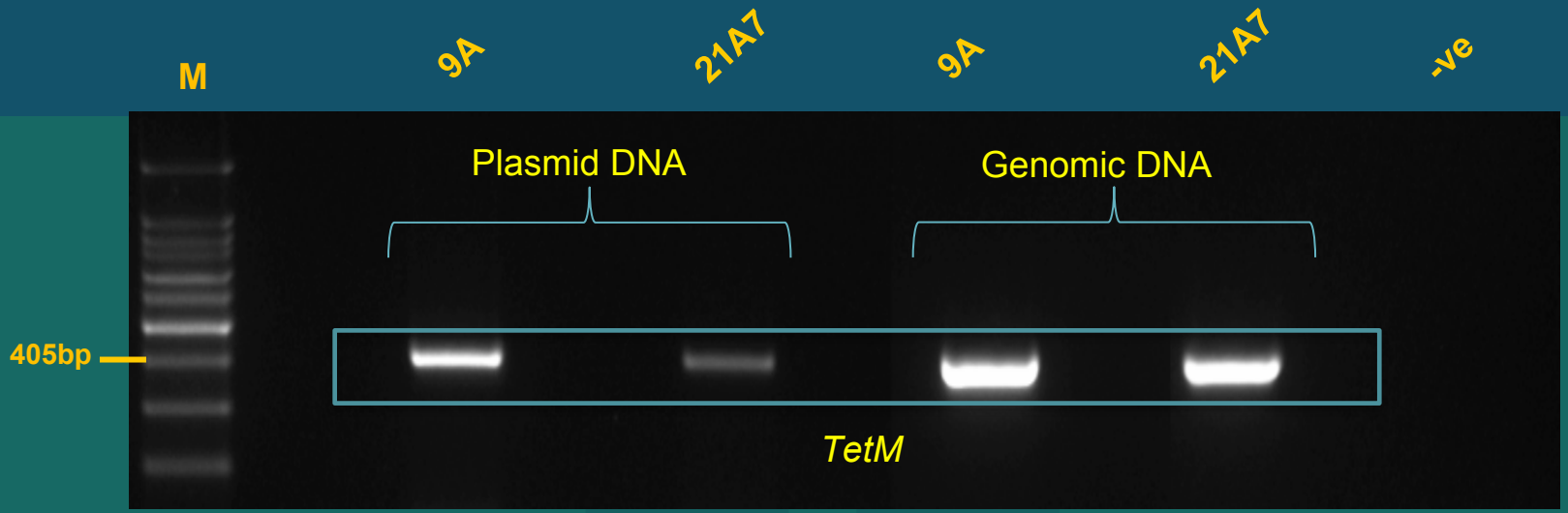
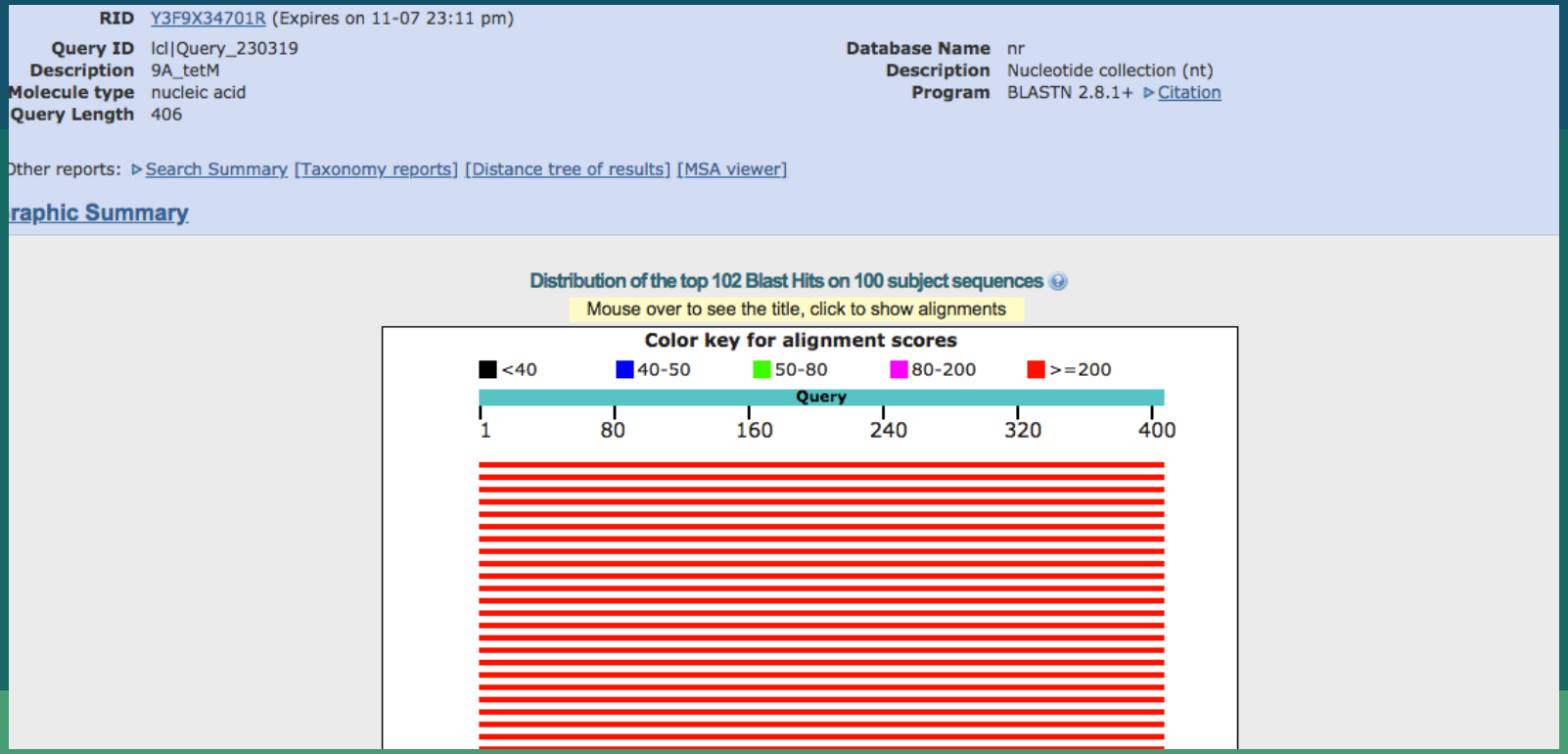


Figure 3 : Multiplex PCR using TetK (614bp), TetL (739bp), TetM (405bp), TetS (589bp) & Int-Tn (525bp) primers were run on 2.5% agarose gel at 90 V for 50 min.

Finding 7: Sequence of TetM Amplicon



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 → acquired tetracycline resistance

L. innocua

1 isolate → acquired tetracycline resistance

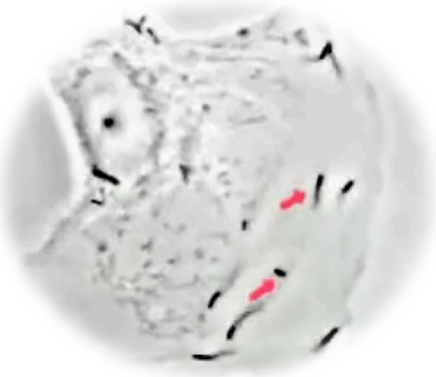
1 isolate → ampicillin & penicillin resistance

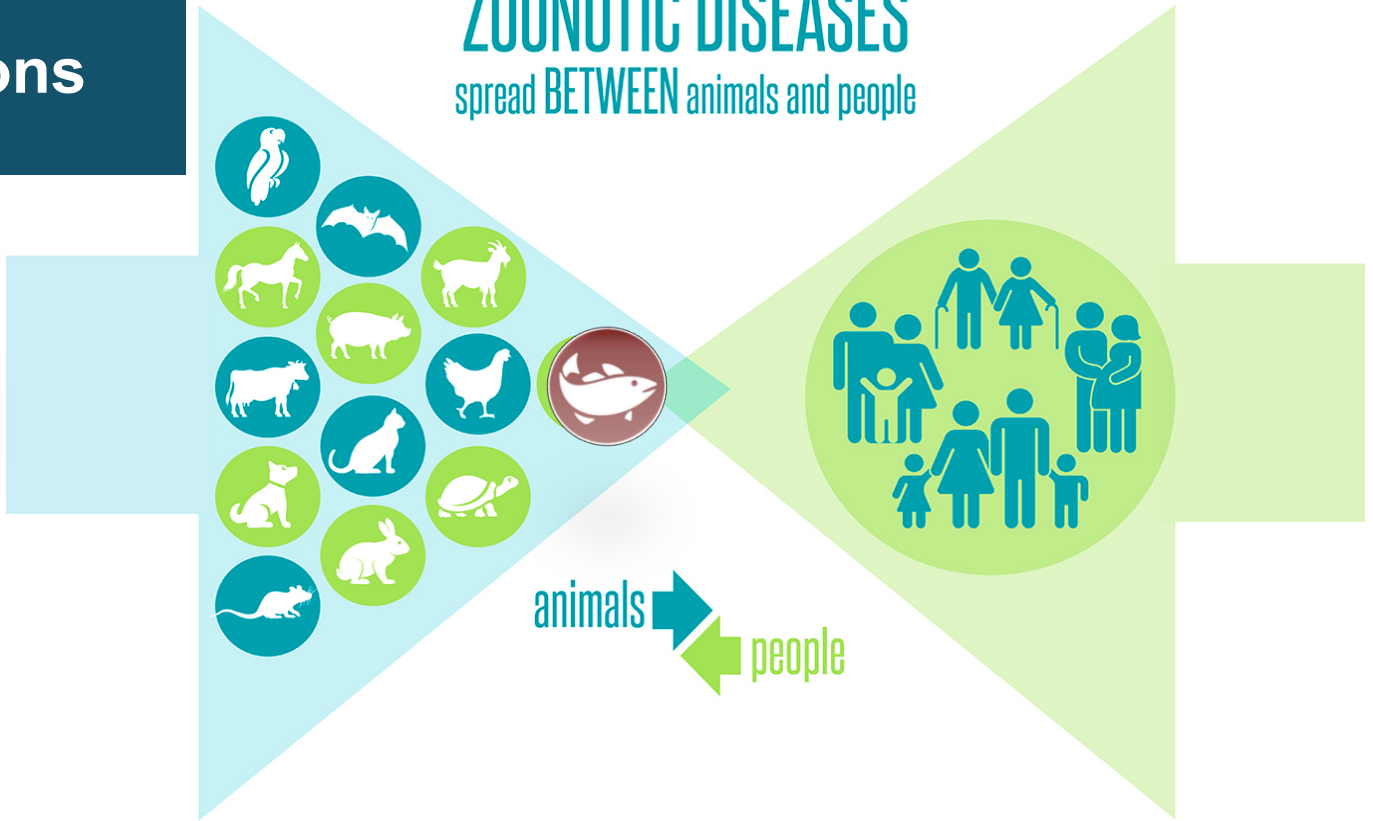
Unidentified *Listeria* spp.

1 isolate → bacitracin resistance

1 isolate → bacitracin resistance & streptomycin

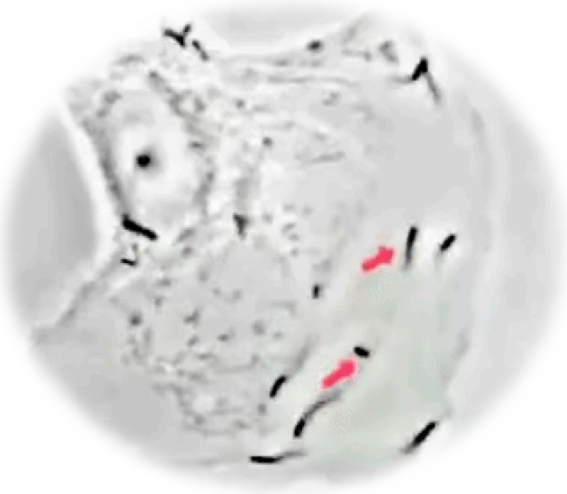
Implications

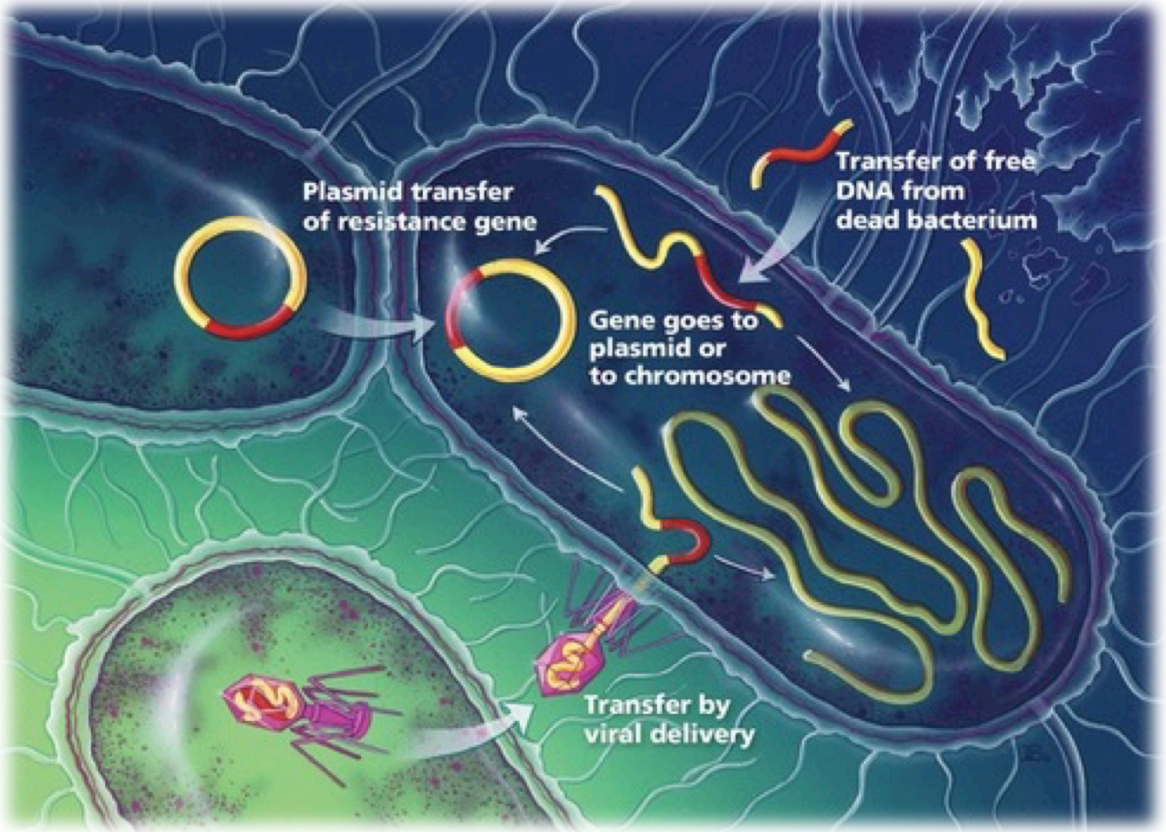




Isolation of *Listeria monocytogenes* from fresh water fish proven its destructive potential to cause foodborne zoonotic listeriosis

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

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THANKS!

Any questions?

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