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Getting a handle on *Listeria* in New Zealand – developing a shared whole genome sequence database for food safety applications

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Plant and Food Research: Graham C. Fletcher, Mark Bradbury (and University of Sydney)

University of Surrey: Arnoud van Vliet (international scientific advisor)

ESR Science for Communities

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Plant & Food™ Research
Rangahau Ahumāra Kai

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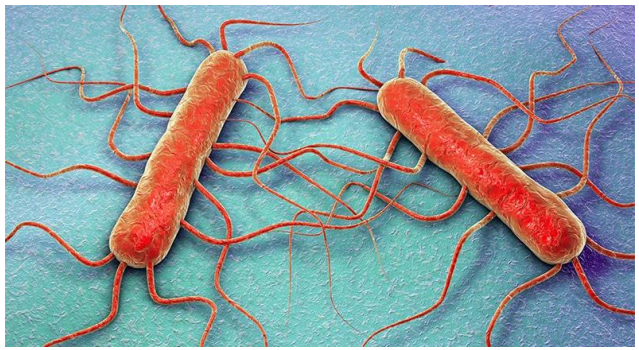
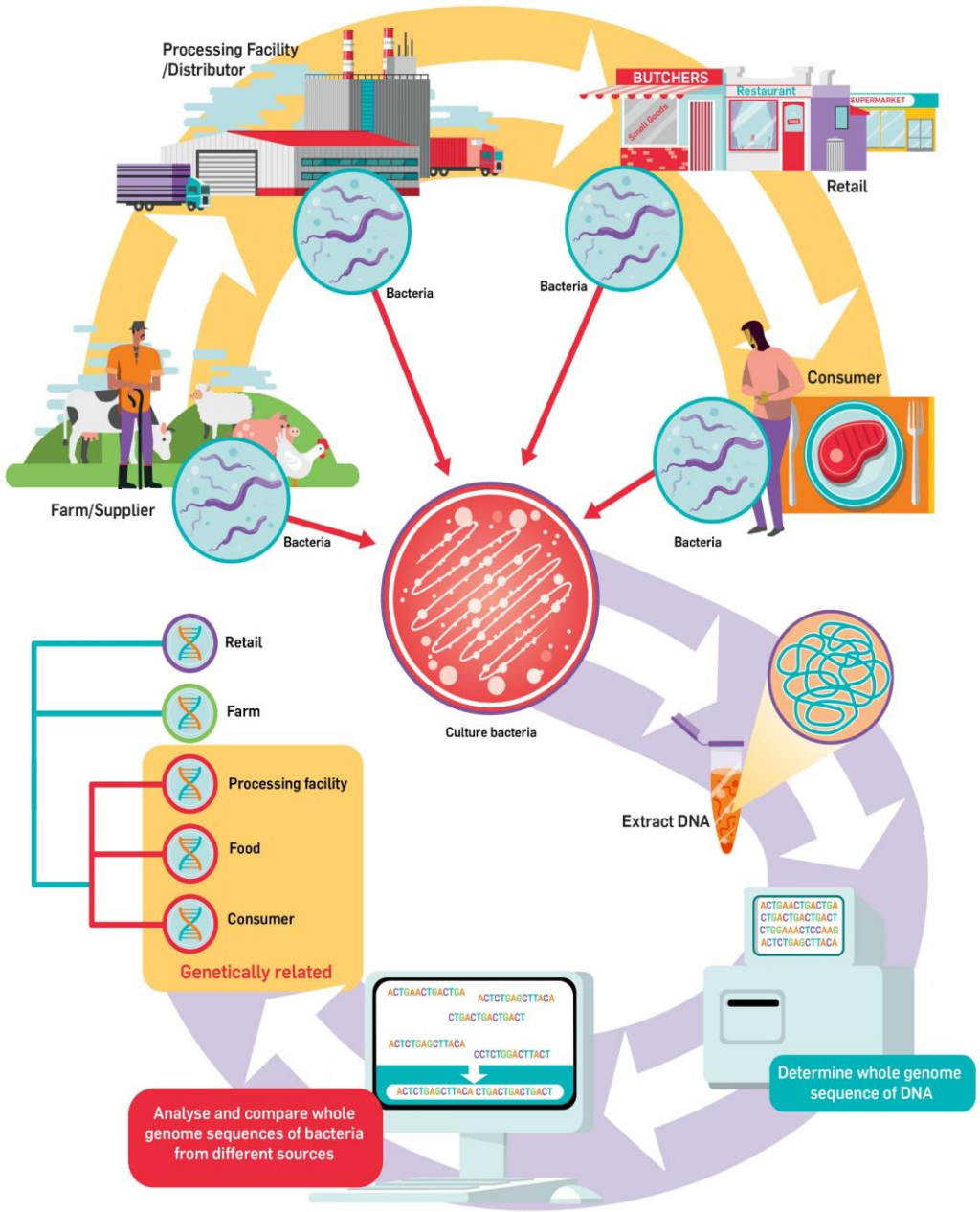
Whole genome sequencing (WGS)

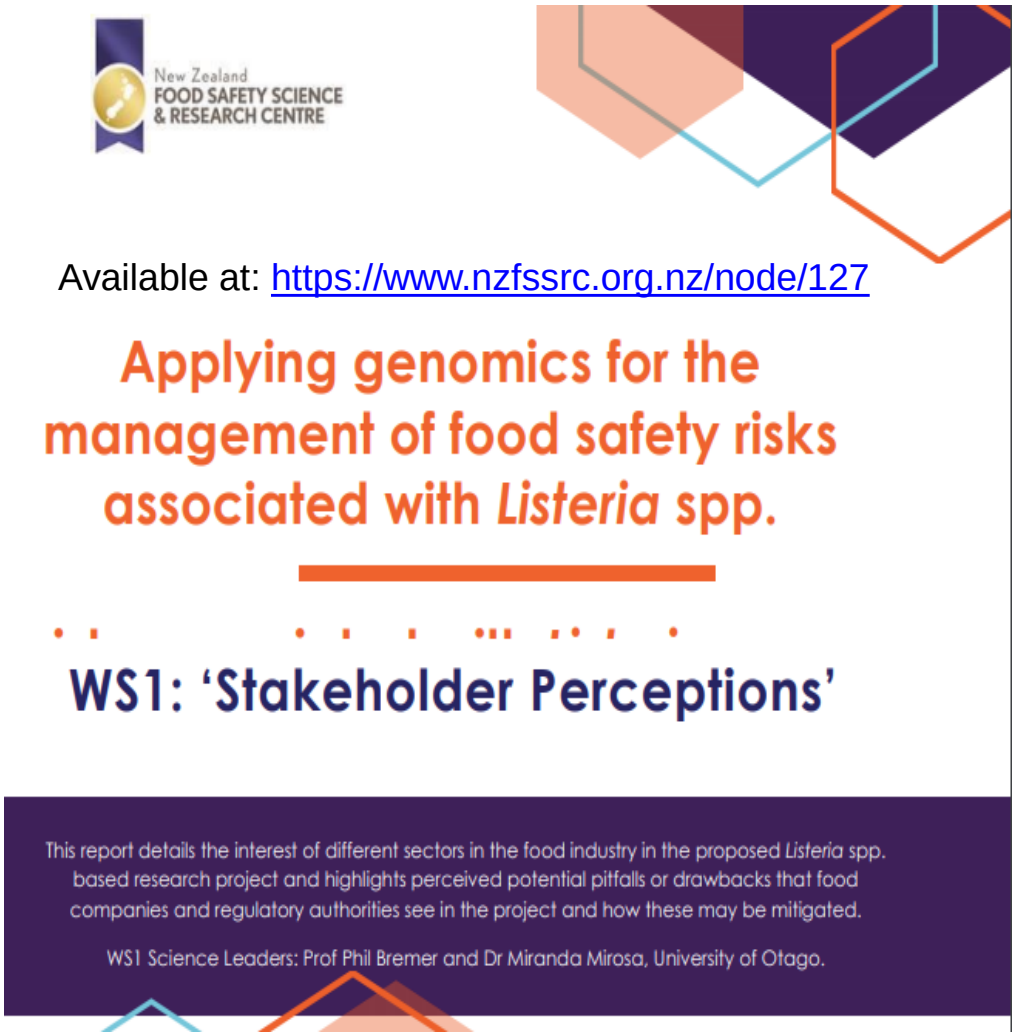
Analysing the entire
(or close to) genome.

- ESR: Sequencing provider
- WGS used throughout the food chain.
- Public health surveillance & outbreaks
- Superseding traditional methods in the laboratory.





NZFSSRC *Listeria* Programme



Available at: https://www.nzfssrc.org.nz/node/127

- Increased awareness of WGS by food industries to solve food safety problems. Therefore generally a positive perception for it's future use.

"I can see benefits. ***Understanding Listeria better,*** *particularly in our raw material supply, and if we can do anything further to* ***minimise those risks"***

"Their [our customers] key drivers are cost and compliance…So anything around food safety is key for them so I would say ***promoting novel technologies for control*** *would be more important to them than for the end consumer"*

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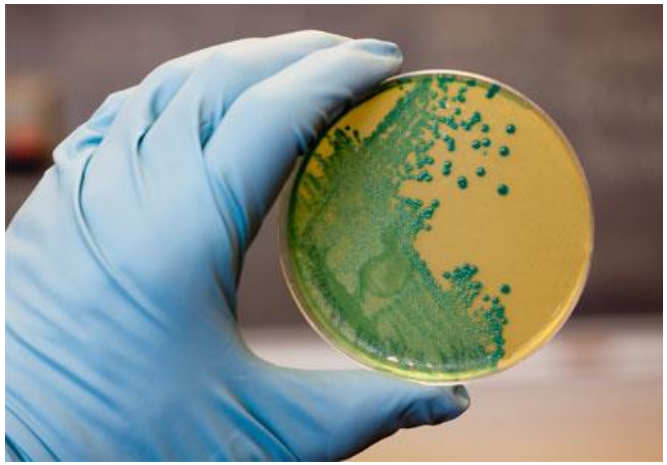
WGS and Industry Uptake

- Greater interest and uptake (helped by NZFSSRC)
- Main questions asked:
  - ❖ Has a cross-contamination event occurred during process into the final product?
  - ❖ Where in the process? E.g. processing water, raw ingredients equipment, staff?
  - ❖ Is it the same to the one found before (weeks, months, years ago?)
  - ❖ How common is this particular strain in NZ?

https://fedequipblog.fedequip.com/the-role-of-equipment-in-the-prevention-of-contamination-in-food-processing/



https://www.foodbusinessnews.net/articles/8991-whole-genome-sequencing-becoming-a-greater-force-in-food-safety

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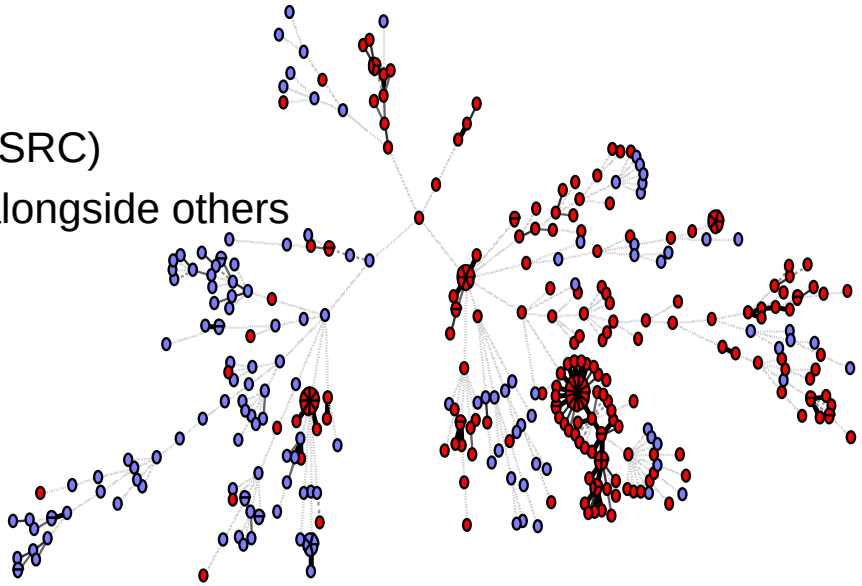
WGS and Industry Uptake

- Educating industry of what WGS results mean and the benefits of WGS is an ongoing task
- It is not straightforward (trees, SNP, alleles/loci, how many? etc)
- As a company's dataset grows…to hundred's of isolates
  - Can you please compare that isolate with all our other isolates?
  - Can we compare with other isolates from NZ?
  - Static images are becoming difficult to generate

- Need to create a WGS resource
  - Industry and scientists can use (NZFSSRC)
  - View and analyse isolates of interest alongside others

Develop a database of WGS data for *Listeria.*



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Shared database for WGS data?

Ministry of Health (clinical)

Ministry for Primary Industries (regulators)

Food Industry/companies

Each research collaborator has *Listeria* WGS data and/or isolates

NZFSSRC *Listeria* WGS Database

Custodians

Consultation of the 'owners/stakeholders' to share the data and conditions of use

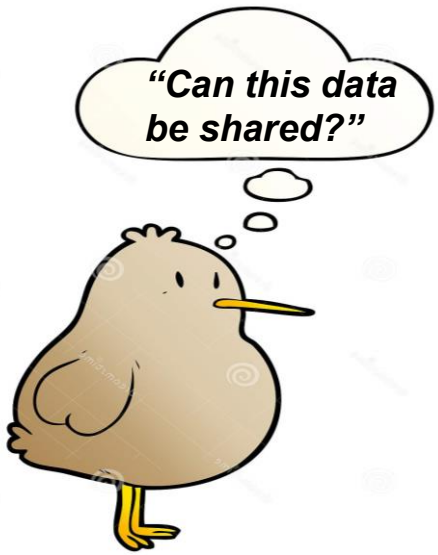


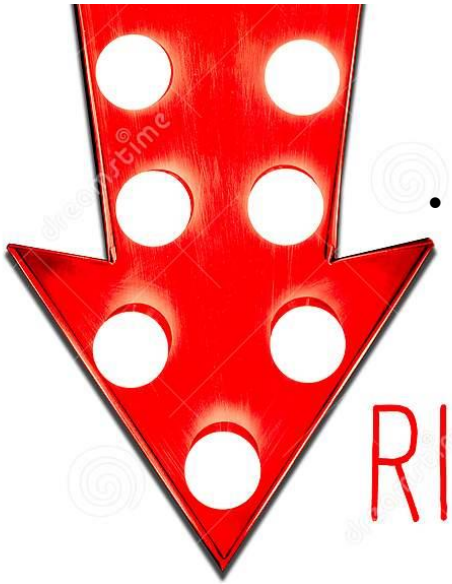
Image: dreamtimes.com

Image: journalofethics.ama-assn.org/article/effective-ways-communicate-risk-and-benefit/2013-01

BENEFIT

- Cross-sector information – wide range of strains from different companies around NZ.
- Understand the diversity, commonalities and persistence of strains in the environment.
- Facilitate knowledge and best practice sharing across industries.

"….how prevalent is the strain? Is this common in the food industry? It is everywhere – don't worry about it …. ***Access to industry wide database is very, very useful to me"***



RISK

- Concern around accessibility of the WGS data.

"….if we develop WGS info and have that stored somewhere that people can access then it makes it ***much easier to lay blame at someone's feet****…"*

- Commercial risk (e.g. reputational damage of being implicated in previous illness).

"If WGS data is compared to clinical data that could then be ***linked back to us****…that's not good"*

Images: dreamtimes.com

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How do you minimise the risk and promote sharing data?

- **Educate and advocate the benefits of WGS and sharing data.**
  - Ongoing discussion and demonstration of how it works.
  - Routinely used resource

- **Limited the level of metadata to be shared.**
  - Unique ID, species, country [NZ], year
  - **Clinical/Non-clinical** (no specific details on foods/sectors).
  - Multi-locus sequence type (ST), core genome allele profile etc (no genomes).
  - Curated by ESR: controlled and standardised (quality control).
  - NZFSSRC users only.

Image: https://www.zerotothree.org/resources/

Image: https://dma.org.uk/article/data-sharing-the-pros-and-cons

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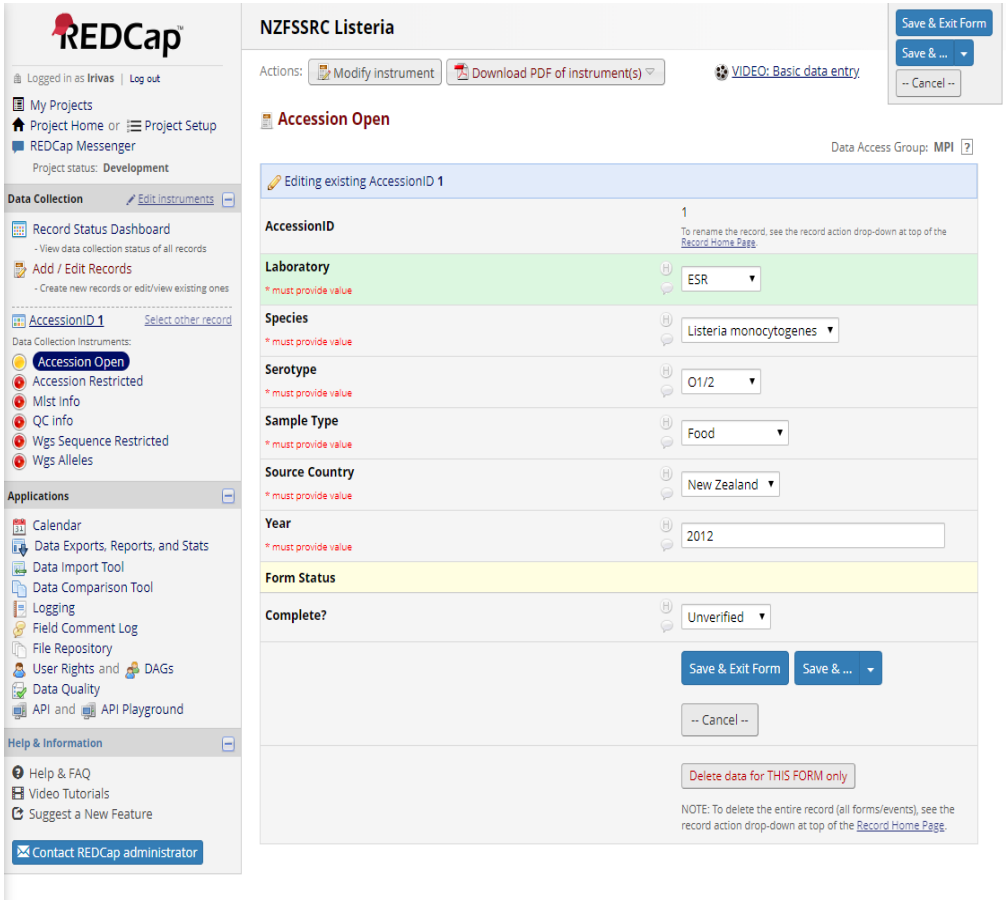
How do you minimise the risk and promote sharing data?

Ensuring data is secure and permissions obtained

- Research Electronic Data Capture (REDCap) system https://www.project-redcap.org/.
- Can create “Data Access Groups” and assign user rights and restrictions to view selected information.



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Limitations to REDCap

- *REDCap only houses data – it can not present WGS data in a useful way nor can create visualisation such as phylogenetic trees.*
  - Does provide an Application Programming Interface (API) key so that it can connect with other applications.

- *REDCap access rights not fit for purpose.*
  - Can restrict an entry (isolate) but not parts of that entry (easily).

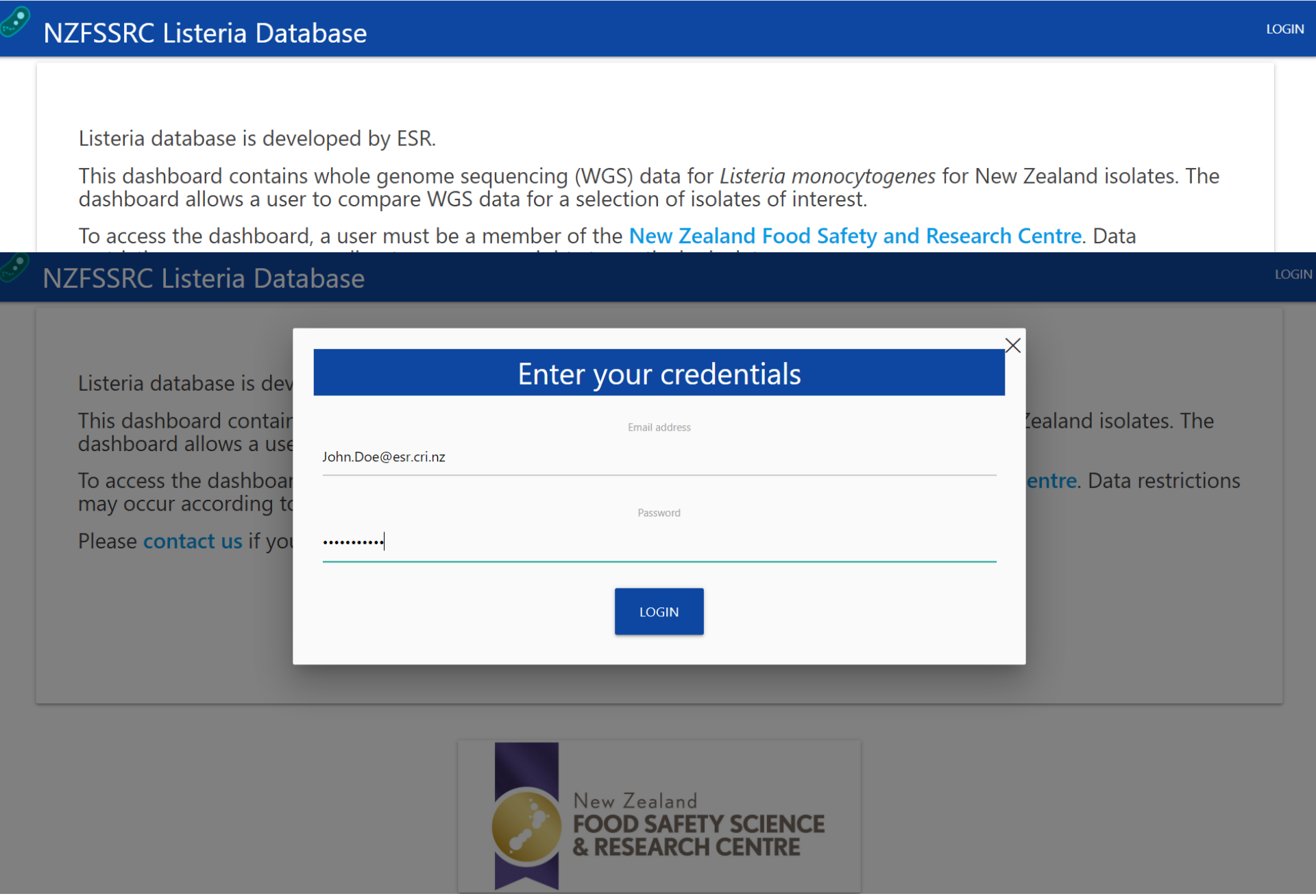
- Challenge of finding alternative solutions whilst ensuring the data remains secure.

- Front end – JavaScript application using React (https://reactjs.org/).

- Backend - Python Flask (https://flask.palletsprojects.com/en/1.1.x/).

- Authentication login – username and password (linked to REDCap).

ıquirylearning428.weebly.com/limitations-of-inquiry-based-learning.html


NZFSSRC Listeria Database
LOGIN
Listeria database is developed by ESR.
This dashboard contains whole genome sequencing (WGS) data for *Listeria monocytogenes* for New Zealand isolates. The dashboard allows a user to compare WGS data for a selection of isolates of interest.
To access the dashboard, a user must be a member of the New Zealand Food Safety and Research Centre. Data
NZFSSRC Listeria Database
LOGIN
Enter your credentials
Email address
John.Doe@esr.cri.nz
Password
LOGIN
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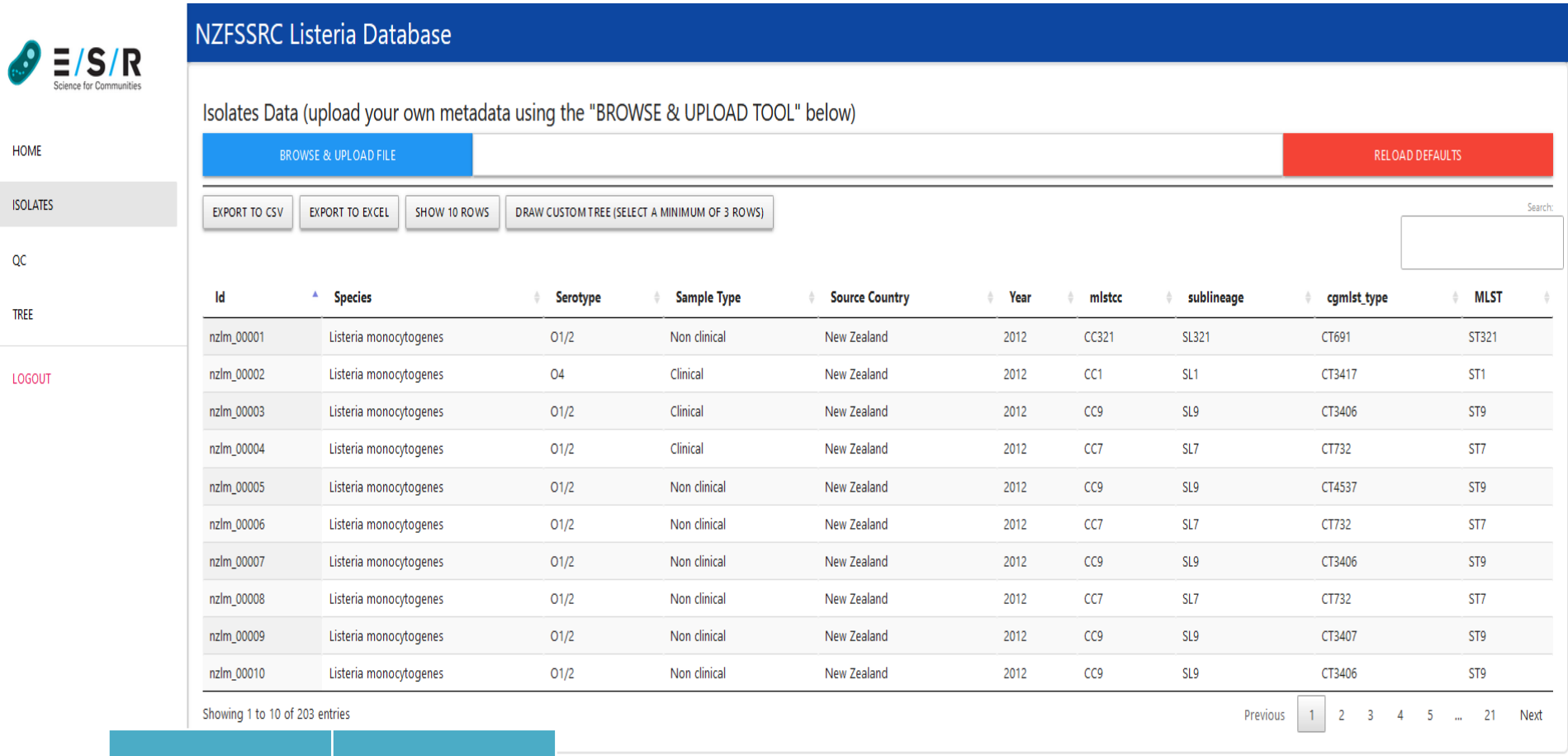
Once logged in then a table listing all isolates in the database will appear with metadata.



Sample Type	Number of isolates to date
Clinical	466
Non-clinical	437
Grand Total	903

- Basic meta-data only for all users (clinical and non-clinical)

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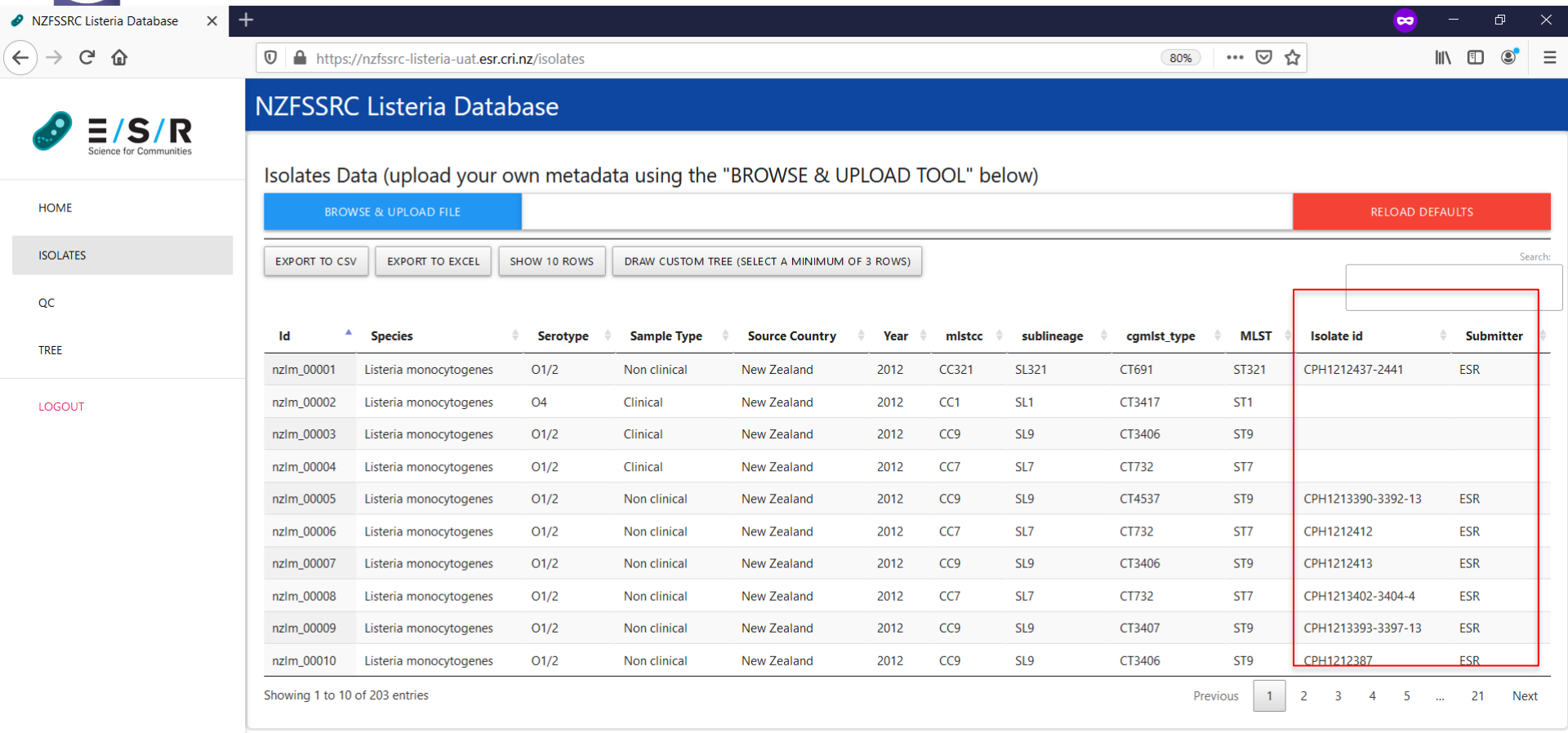
A specific user login view



- Client identifiers only in view for that client/approved users.
- This helps a client identify their isolates amongst the others.
- Working towards making a ‘collection’ for a particular user (possibility to make entries completely private)

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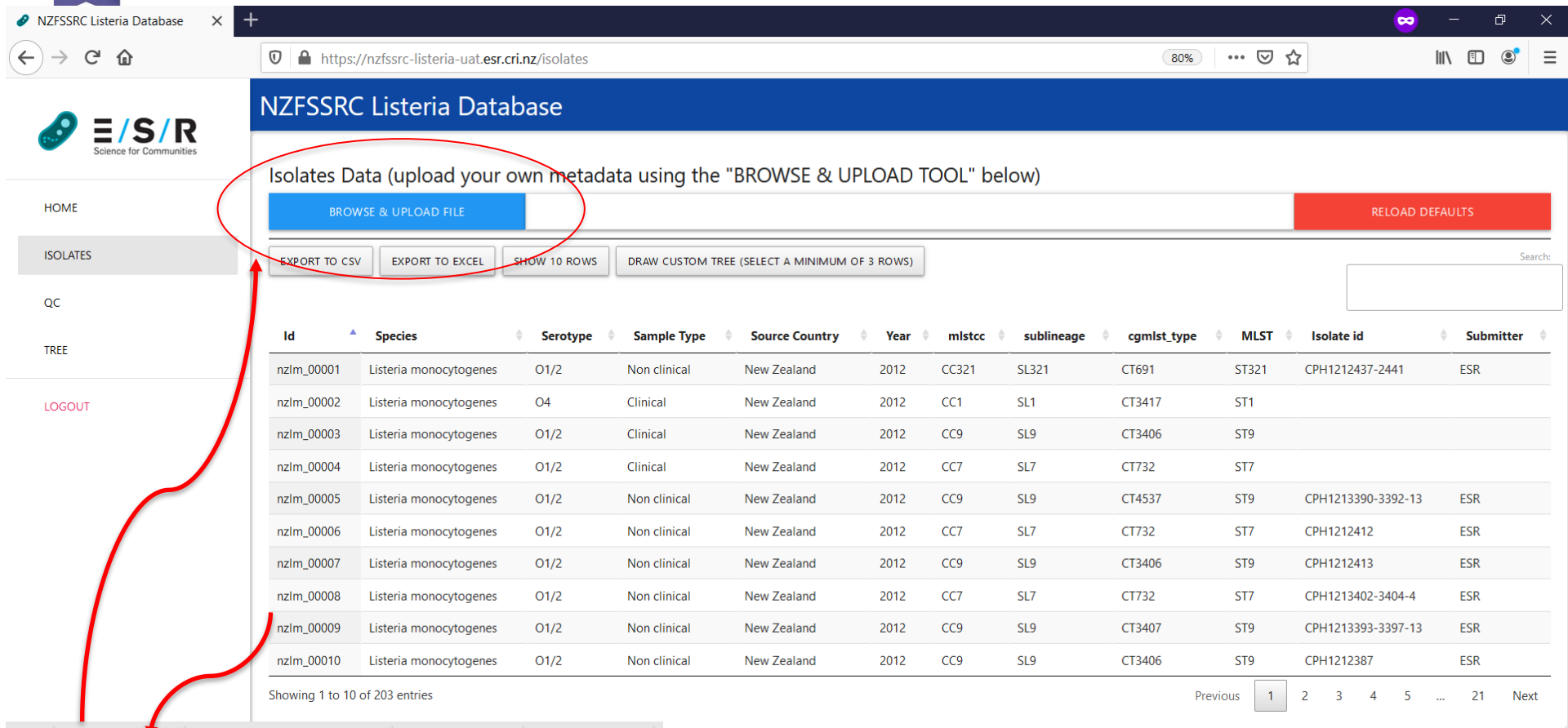

	A	B	C	D
1	Id	Location	Date	
2	5	Packer	2020	
3	7	Raw ingredient	2021	
4	9	Slicer	2021	
5	10	Final product	2021	
6				
7				

- Additional user data about an isolate can be uploaded to a session (Excel).

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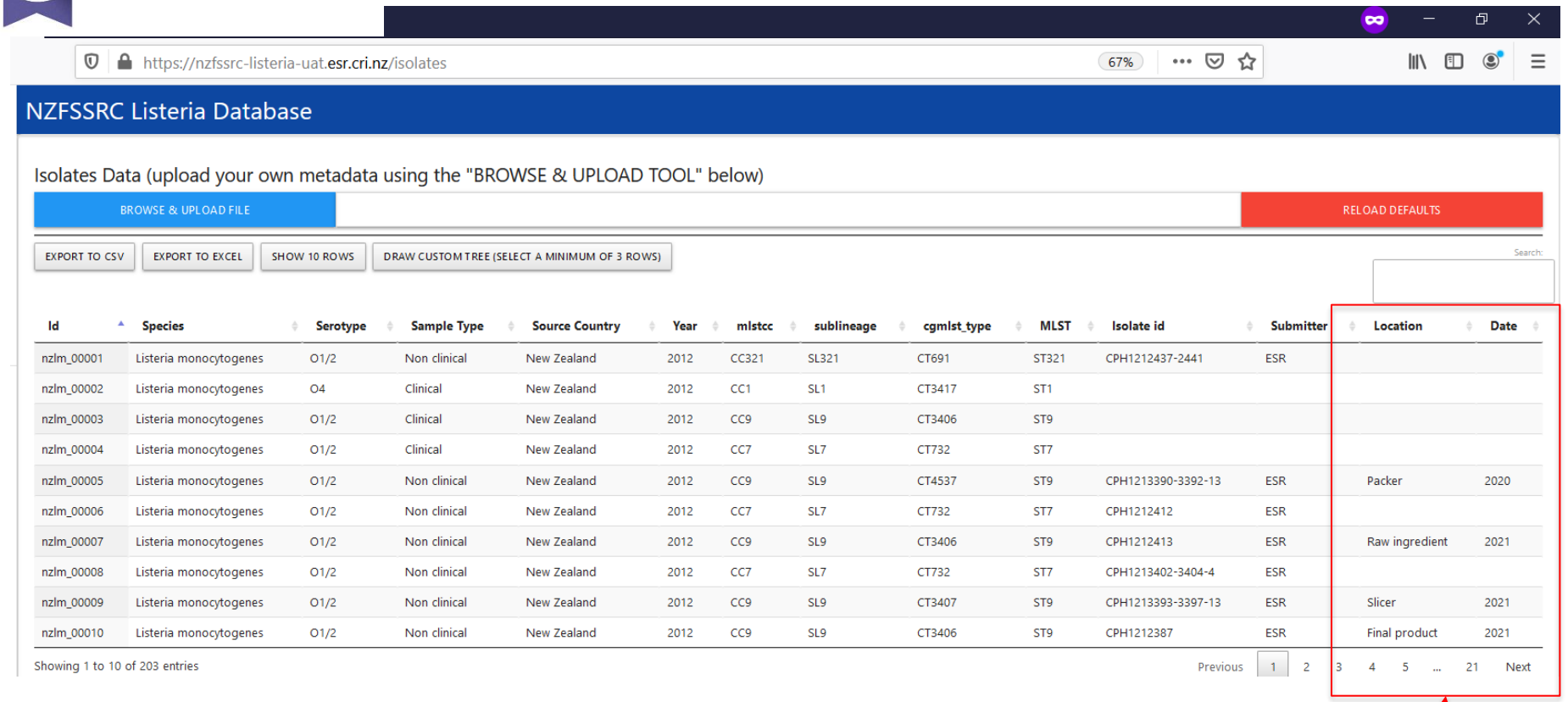

Id	Species	Serotype	Sample Type	Source Country	Year	mlstcc	sublineage	cgmlst_type	MLST	Isolate id	Submitter	Location	Date
nzlm_00001	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC321	SL321	CT691	ST321	CPH1212437-2441	ESR		
nzlm_00002	Listeria monocytogenes	O4	Clinical	New Zealand	2012	CC1	SL1	CT3417	ST1				
nzlm_00003	Listeria monocytogenes	O1/2	Clinical	New Zealand	2012	CC9	SL9	CT3406	ST9				
nzlm_00004	Listeria monocytogenes	O1/2	Clinical	New Zealand	2012	CC7	SL7	CT732	ST7				
nzlm_00005	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT4537	ST9	CPH1213390-3392-13	ESR	Packer	2020
nzlm_00006	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC7	SL7	CT732	ST7	CPH1212412	ESR		
nzlm_00007	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3406	ST9	CPH1212413	ESR	Raw ingredient	2021
nzlm_00008	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC7	SL7	CT732	ST7	CPH1213402-3404-4	ESR		
nzlm_00009	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3407	ST9	CPH1213393-3397-13	ESR	Slicer	2021
nzlm_00010	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3406	ST9	CPH1212387	ESR	Final product	2021

- Extra user information is added to the meta-data table and also follows the analysis (tree).
- Not saved data
- Deleted after the session ends

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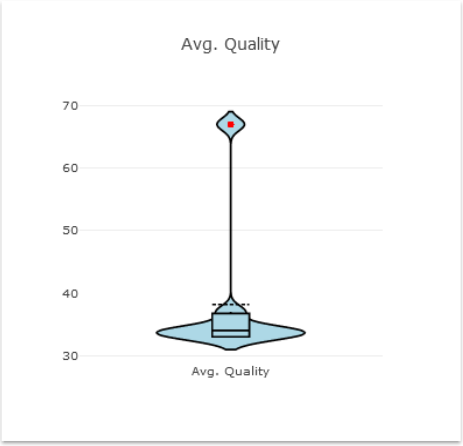
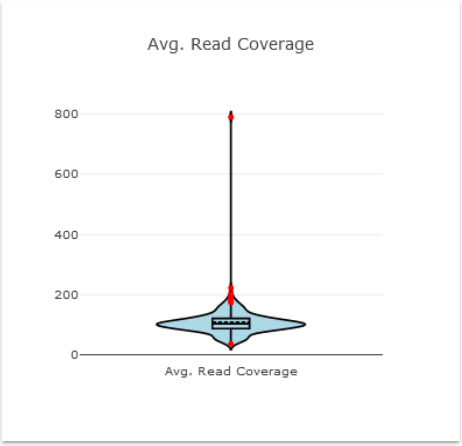
Quality Control Statistics

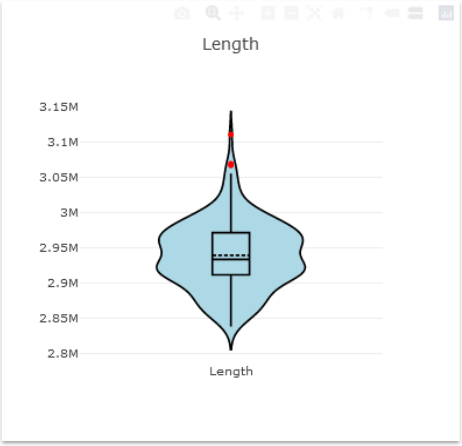
Id	Avg. Quality	Avg. Read Coverage	Length	N50	# BAF Perfect	# BAF Present	# Bases ACGT	# Contigs
1	67	121	3068674	477952	3004	3132	3059237	20
2	67	104	2990762	475319	2859	3046	2975273	28
3	67	99	2946161	604853	2904	3019	2939704	15
4	67	101	2876624	1458953	2815	2936	2871279	14
5	67	84	2948865	449139	2917	3030	2943356	19
6	67	202	2876044	1458346	2815	2937	2872490	16
7	67	191	2946098	604853	2908	3021	2940982	13
8	67	168	2876408	1458317	2816	2937	2872507	16
9	67	181	2945799	604826	2907	3019	2940360	14
10	37	51	2970753	449615	2824	3046	2965968	20

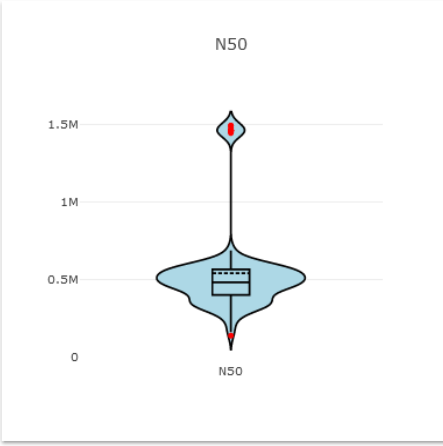
Showing 1 to 10 of 203 entries

Previous 1 2 3 4 5 ... 21 Next









BAF Perfect

BAF present

Bases ACGT

Contigs

ESR is the database curator. Good quality data uploaded only

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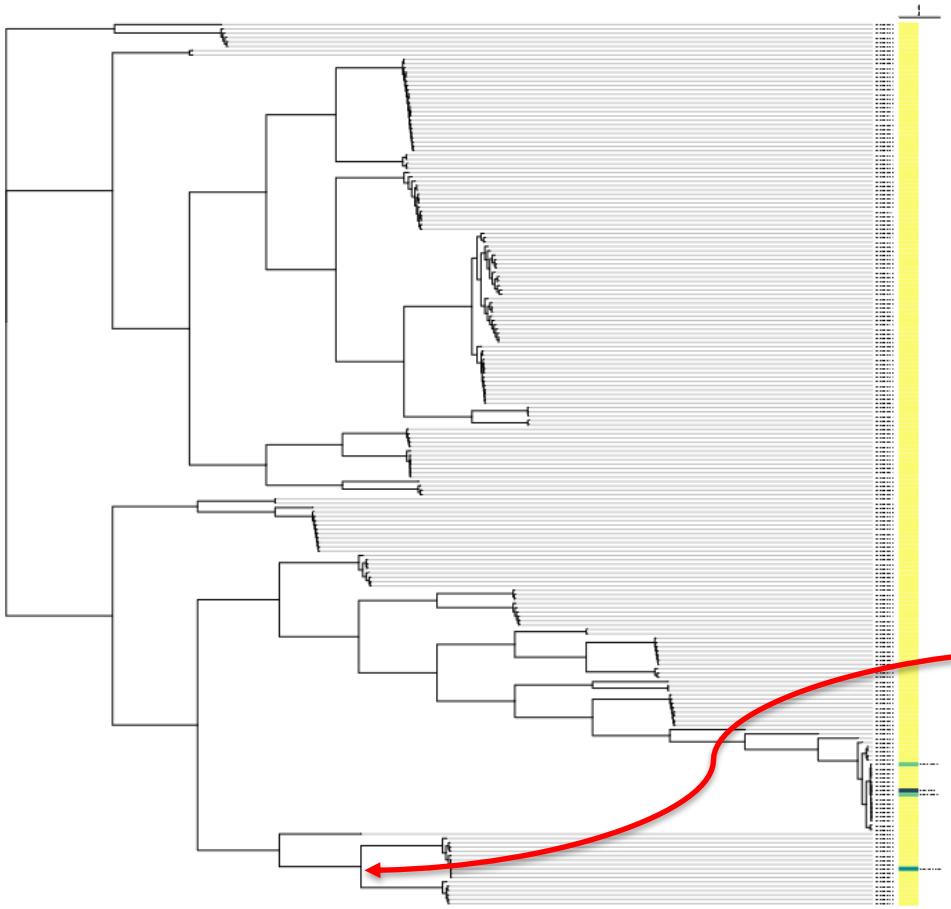
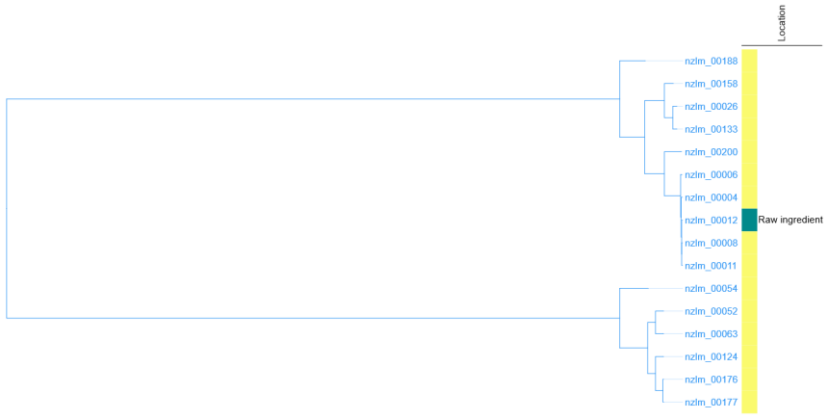
"Trees"

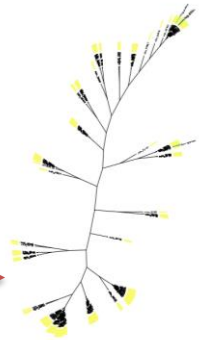
- PhyloCanvas https://phylocanvas.org
- Uses core-genome MLST (>1400 alleles)

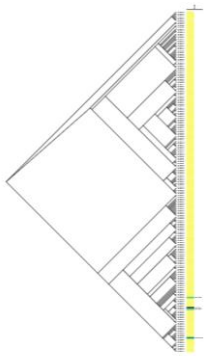


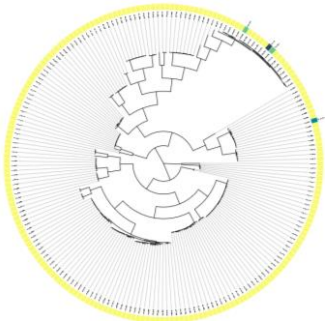
- Default is to load a big tree with all of the isolates.



- Select a node of interest and re-draw the tree (right click)







- Different layouts (radial, circular etc).
- Different labels can be included

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EXPORT TO CSV | EXPORT TO EXCEL | SHOW 20 ROWS | DRAW CUSTOM TREE (SELECT A MINIMUM OF 3 ROWS)

Id	Species	Serotype	Sample Type	Source Country	Year	mlstcc	sublineage	cgmlst_type	MLST	Isolate id	Submitter	Location	Date
nzlm_00007	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3406	ST9	CPH1212413	ESR		
nzlm_00008	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC7	SL7	CT732	ST7	CPH1213402-3404-4	ESR		
nzlm_00009	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3407	ST9	CPH1213393-3397-13	ESR	Final product	2015
nzlm_00010	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3406	ST9	CPH1212387	ESR		
nzlm_00011	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC7	SL7	CT732	ST7	CPH1212392	ESR		
nzlm_00012	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC7	SL7	CT732	ST7	CPH1212393	ESR	Raw ingredient	2021
nzlm_00013	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3406	ST9	CPH1212395	ESR	Floor drain	2021
nzlm_00014	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3406	ST9	CPH1212398-402	ESR	Final product	2021
nzlm_00015	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3406	ST9	CPH1213368-1	ESR		
nzlm_00016	Listeria monocytogenes	O1/2	Non clinical	New Zealand	2012	CC9	SL9	CT3407	ST9	CPH1213369-3371-1	ESR		

Showing 1 to 20 of 203 entries 4 rows selected

Previous 1 2 3 4 5 ... 11 Nex

- Select isolates of interest
- Create a custom tree (easier to visualise a tree with fewer isolates)

Numbers on branches are core allele differences between isolates

- Raw ingredient isolate is not genetically related to the others

2015 isolate less genetically related to 2021 isolates. Mutated over time (6 years)? Re-introduction from a common source?

Additional isolates over time can helpful

- Final product and Floor drain isolates from 2021 share 0 allele differences – genetically closely related – possible shared contamination pathway?

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How do you minimise the risk and promote sharing data?

- The database is not intended to be an epidemiological public health tool.
- Concordance with epidemiological data is essential to link foodborne cases to a suspected food source.
- Restrict clinical data for last 12 months.
- Develop a process where the food processor can work with the regulator (MPI).
- ESR as curators will perform analysis to ‘flag’ potential concerns.

Image: https://devops.com/

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Future work and developments

- Industry engagement and feedback.
  - Fine-tuning the dashboard with user feedback.
  - Potential to creating links for users to map their isolates onto processing plant maps (additional security needed).

- Research into the population structure of *L. monocytogenes* for NZ.

- Incorporate other genetic determinants such as resistance and persistence genes (sanitizers/cleaning agents).

"Is this Listeria in the processing plant resistant to the cleaning being done? What else can be used to eliminate it?"

Acknowledgements

ESR: Phani Atmakur.

University of Otago: Phil Bremer and Miranda Mirosa.

Representatives from the New Zealand Ministry for Primary Industries, Ministry of Health, food companies that were consulted and contributing to data to the database.

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