

Case study 3- the virtual laboratory

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Background to conventional bacteriological approaches versus big data -

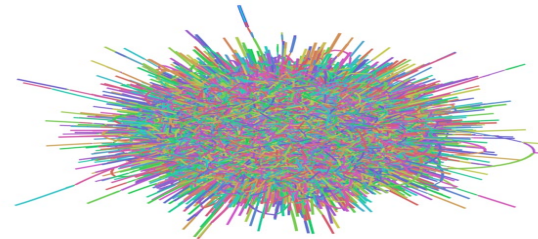
- microbiological safety in food has traditionally been monitored by making use of ***culture-based phenotypic methods***; detection characterisation & identification ***-bed rock of diagnostic bacteriology***
- bacterial pathogens of importance to public health are primarily studied at the **genus/species level** and with no regard to the context of the microbial communities from which they came ***-microbiome***
- ***high-throughput sequencing strategies have heralded a seismic shift*** -study these bacteria of importance to human health and provided the opportunity to ***re-write the risk assessment paradigm***
- ***big data-based analysis is now a reality upon which future food safety risk assessment will be based – Precision Food Safety***



MacConkey agar



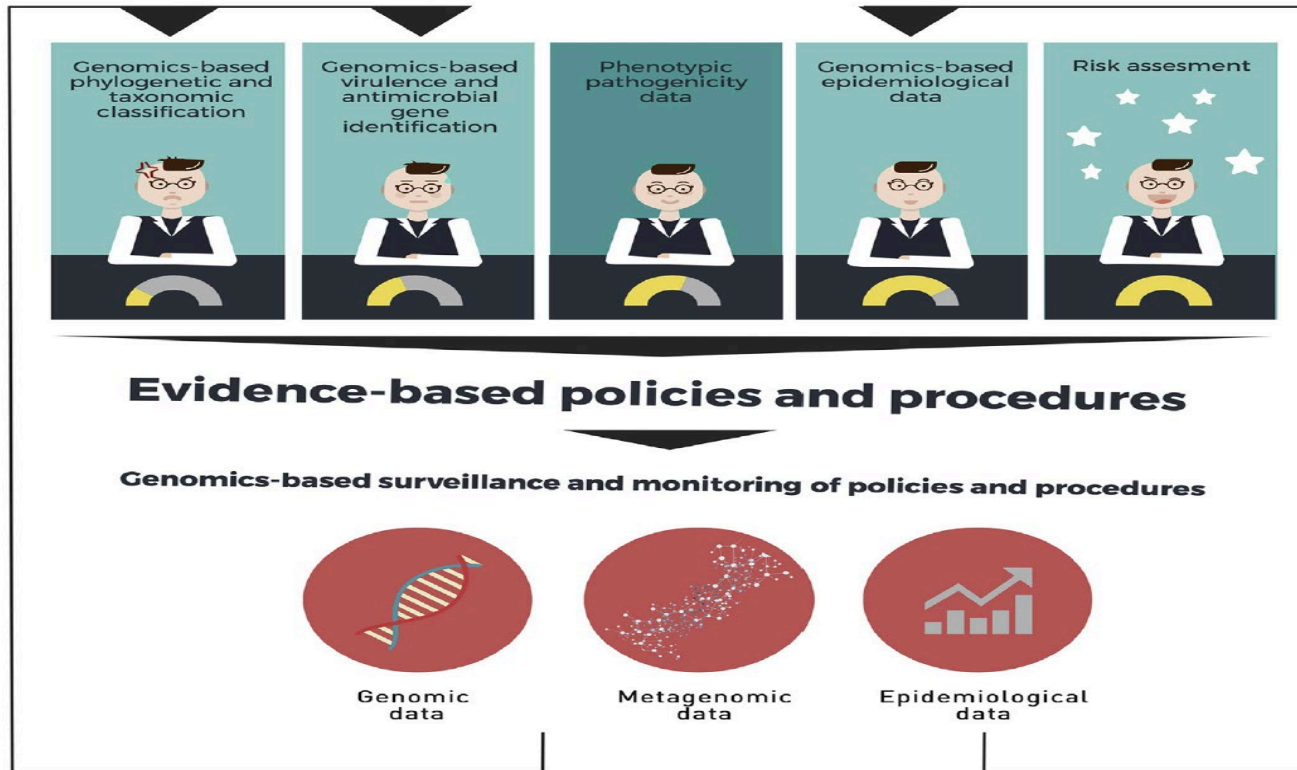
Blood agar



Whole genome sequencing



Precision food safety -



WGS-based analytics related to precision food safety -

- **Identify source of food-borne outbreaks quickly -**
 - Whole Genome Sequencing (WGS) surveillance of bacterial pathogens-tracking & tracing worldwide;
 - Environmental monitoring;
 - Elucidate features of importance to food safety (protection of the consumer/brand reputation) and food quality;
- **Detection of food fraud-**
 - Species identification;
 - Products of designated origin (PDO);
- **Risk assessment – a working hypothesis!**
 - Using *big (genome) data* can we differentiate between a (regulatory controlled) pathogen and one that is *a true pathogen?*
 - *This is the shape of risk assessment into the future*

Objectives-

- **Development of harmonised SOPs with project partners**
 - Bacterial culturing
 - DNA purification and quality assessment
 - Sequencing protocol
 - Whole Genome Sequencing (WGS)
- **Evaluation of the performance - exercise with 24 isolates**
 - bioinformatics pipelines & SNP analysis
- **SARS-CoV-2 collaboration**



Schematic representation of the WGS bioinformatics pipeline -

seafood



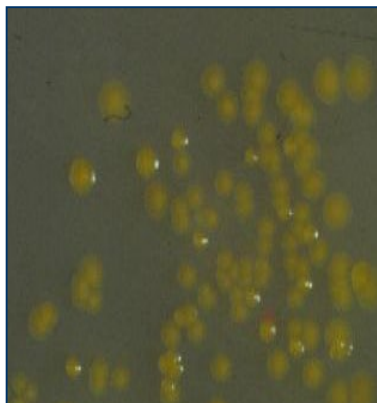
Listeria monocytogenes

poultry & pig meat



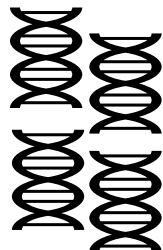
Salmonella Typhimurium

powder infant formula



Cronobacter sakazakii

DNA purification



Library preparation



Sequencing



Raw reads



Raw reads

Read processing
QA/QC

De novo
assembly

Epi-info

Species confirmation
detection

Phylogenetic
inference

Maximum likelihood
and Bayesian methods

Distance
methods

Minimum
spanning tree

Allele
calling

cg/wgMLST

Reference-based
mapping (SNP)

De novo allele
calling (Kmer)

In silico
phenotype

Serotyping

Resistome

Virulome

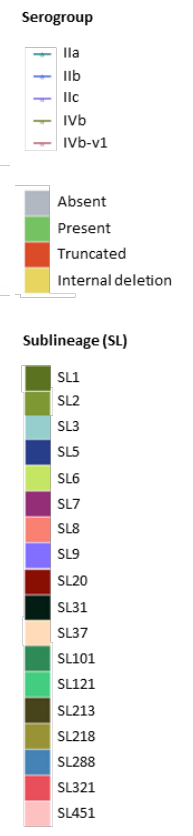
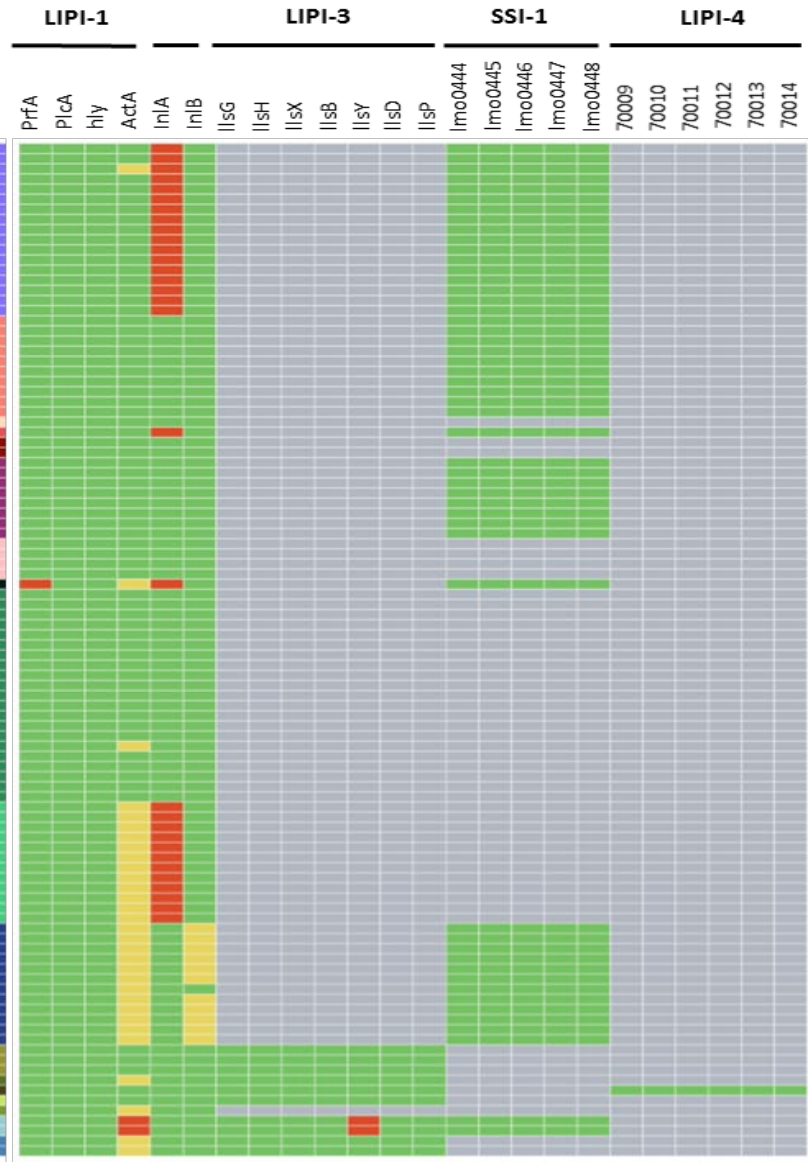
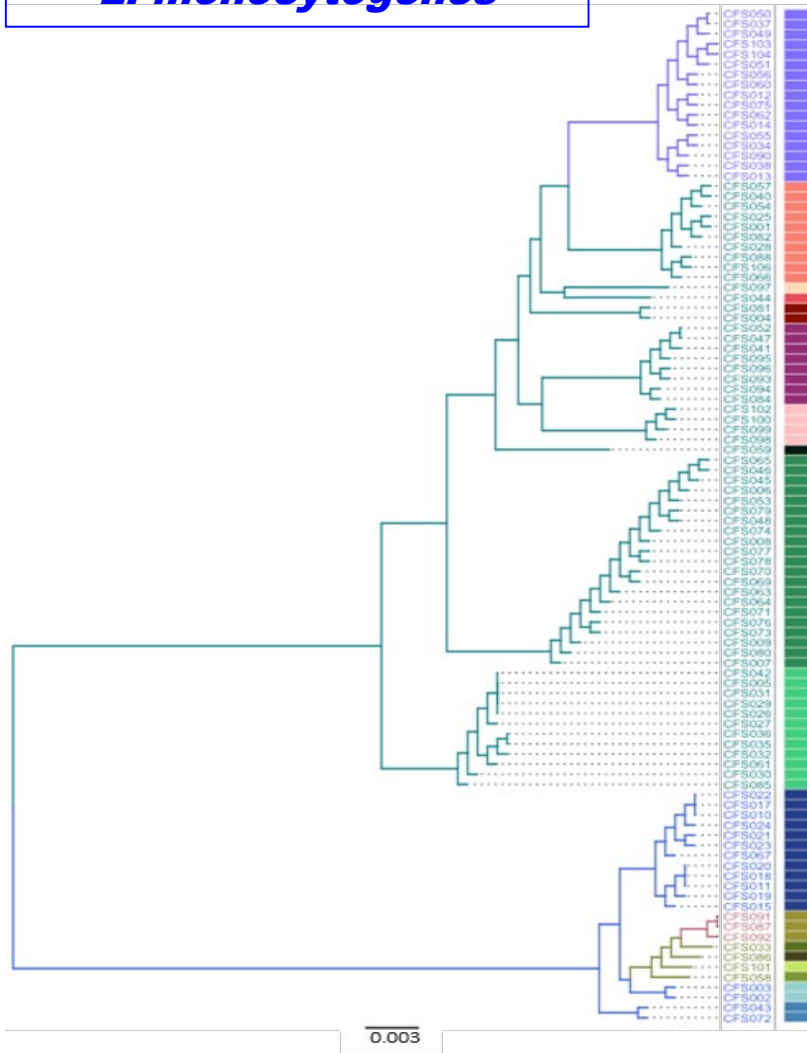


Identification of the test bacterium based on WGS analysis -

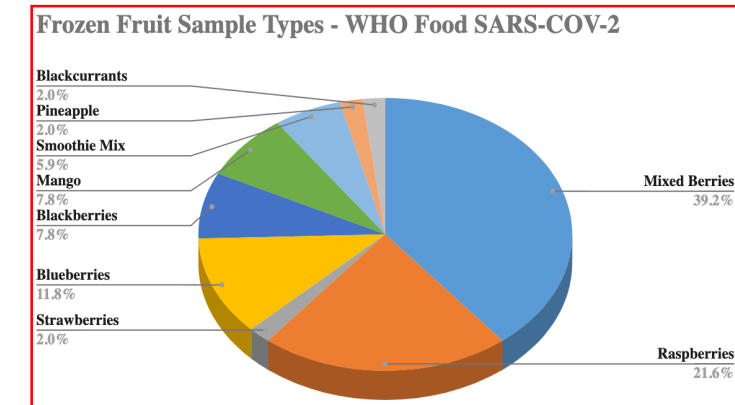
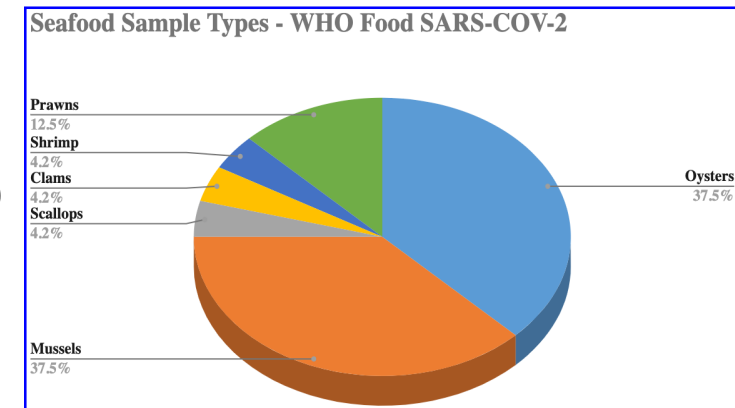
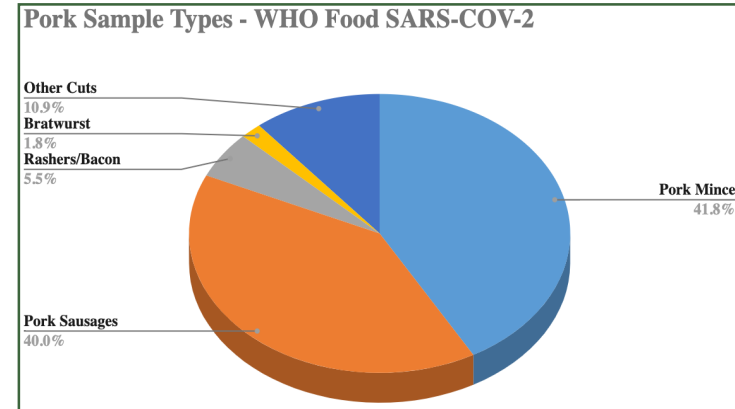
identification and typing													
n isolate	Strain	MLST scheme							Salmonella Predicted antigenic profile	O	H1 (fliC)	H2 (fliB)	Predicted serotype
1	CFS3535	atpD(126)	fusA(73)	glnS(~116)	gltB(~111)	gyrB(~111)	infB(~112)	pps(~142)	N.A.	N.A.	N.A.	N.A.	N.A.
2	CFS3536	atpD(5)	fusA(1)	glnS(3)	gltB(3)	gyrB(5)	infB(5)	pps(4)	N.A.	N.A.	N.A.	N.A.	N.A.
3	CFS3537	atpD(126)	fusA(73)	glnS(~116)	gltB(~111)	gyrB(~111)	infB(~112)	pps(~142)	N.A.	N.A.	N.A.	N.A.	N.A.
4	CFS3538	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
5	CFS3539	atpD(5)	fusA(1)	glnS(3)	gltB(3)	gyrB(5)	infB(5)	pps(4)	N.A.	N.A.	N.A.	N.A.	N.A.
6	CFS3540	atpD(5)	fusA(1)	glnS(3)	gltB(3)	gyrB(5)	infB(5)	pps(4)	N.A.	N.A.	N.A.	N.A.	N.A.
7	CFS3541	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	CFS3542	atpD(5)	fusA(1)	glnS(3)	gltB(3)	gyrB(5)	infB(5)	pps(4)	N.A.	N.A.	N.A.	N.A.	N.A.
9	CFS4391	aroC(15)	dnaN(70)	hemD(93)	hisD(78)	purE(113)	sucA(6)	thrA(68)	7:z10:e,n,z15	O-7	z10	e,n,z15	Mbandaka
10	CFS4392	aroC(15)	dnaN(70)	hemD(93)	hisD(78)	purE(113)	sucA(6)	thrA(68)	7:z10:e,n,z15	O-7	z10	e,n,z15	Mbandaka
11	CFS4393	aroC(15)	dnaN(70)	hemD(93)	hisD(78)	purE(113)	sucA(6)	thrA(68)	7:z10:e,n,z15	O-7	z10	e,n,z15	Mbandaka
12	CFS4394	aroC(15)	dnaN(70)	hemD(93)	hisD(78)	purE(113)	sucA(6)	thrA(68)	7:z10:- N/A	O-7	z10	-	N/A*
13	CFS4395	aroC(15)	dnaN(70)	hemD(93)	hisD(78)	purE(113)	sucA(6)	thrA(68)	7:z10:e,n,z15	O-7	z10	e,n,z15	Mbandaka
14	CFS4396	aroC(15)	dnaN(70)	hemD(93)	hisD(78)	purE(113)	sucA(6)	thrA(68)	7:z10:e,n,z15	O-7	z10	e,n,z15	Mbandaka
15	CFS4397	aroC(15)	dnaN(70)	hemD(93)	hisD(78)	purE(113)	sucA(6)	thrA(68)	7:z10:e,n,z15	O-7	z10	e,n,z15	Mbandaka
16	CFS4398	aroC(15)	dnaN(70)	hemD(93)	hisD(78)	purE(113)	sucA(6)	thrA(68)	7:z10:e,n,z15	O-7	z10	e,n,z15	Mbandaka
17	F2151	abcZ(7)	bglA(6)	cat(8)	dapE(8)	dat(6)	ldh(37)	lhkA(1)	N.A.	N.A.	N.A.	N.A.	N.A.
18	F2152	abcZ(7)	bglA(6)	cat(8)	dapE(8)	dat(6)	ldh(37)	lhkA(1)	N.A.	N.A.	N.A.	N.A.	N.A.
19	F2153	abcZ(7)	bglA(6)	cat(8)	dapE(8)	dat(6)	ldh(37)	lhkA(1)	N.A.	N.A.	N.A.	N.A.	N.A.
20	F2154	abcZ(7)	bglA(6)	cat(8)	dapE(8)	dat(6)	ldh(37)	lhkA(1)	N.A.	N.A.	N.A.	N.A.	N.A.
21	F2155	abcZ(7)	bglA(6)	cat(8)	dapE(8)	dat(6)	ldh(37)	lhkA(1)	N.A.	N.A.	N.A.	N.A.	N.A.
22	F2160	abcZ(7)	bglA(6)	cat(8)	dapE(8)	dat(6)	ldh(37)	lhkA(1)	N.A.	N.A.	N.A.	N.A.	N.A.
23	F2161	abcZ(7)	bglA(6)	cat(8)	dapE(8)	dat(6)	ldh(37)	lhkA(1)	N.A.	N.A.	N.A.	N.A.	N.A.
24	F2166	abcZ(2)	bglA(1)	cat(11)	dapE(3)	dat(3)	ldh(1)	lhkA(7)	N.A.	N.A.	N.A.	N.A.	N.A.



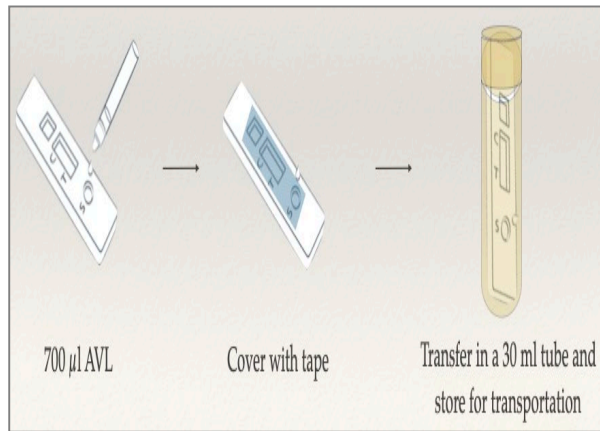
Re-defining risk to human health based on WGS data for *L. monocytogenes*



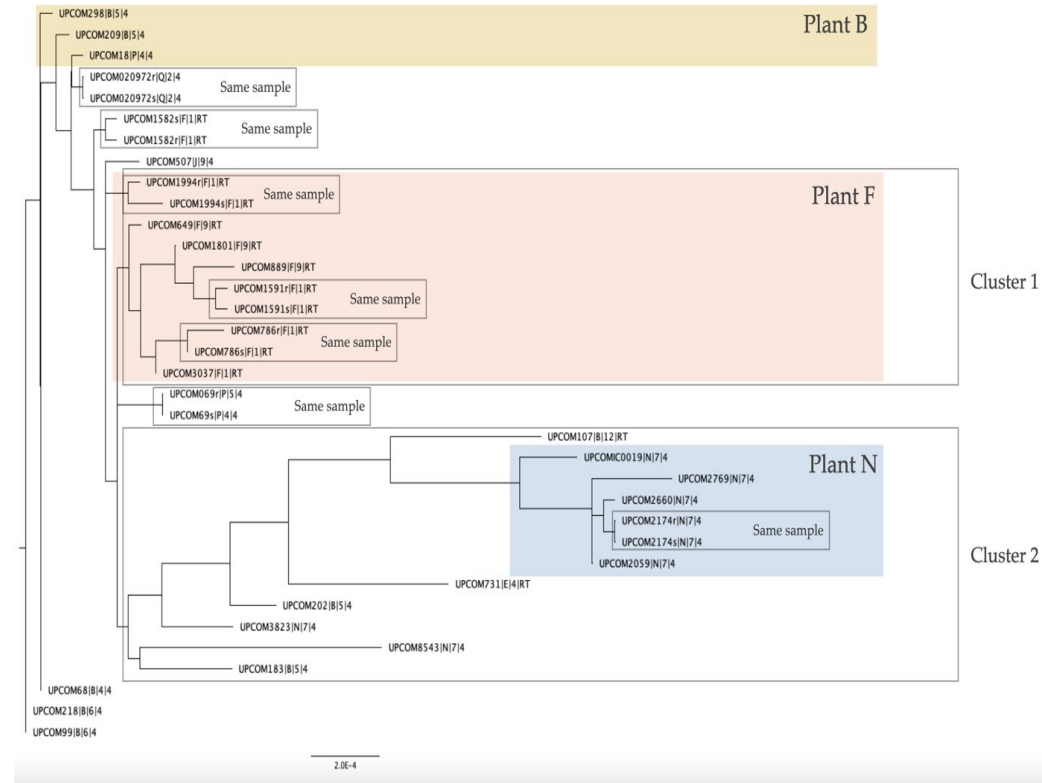
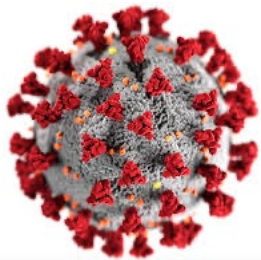
SARS-CoV-2 and food security -



Extending the diagnostic value of spent rapid antigen detection assay kits in a meat processing plant (MPP) -

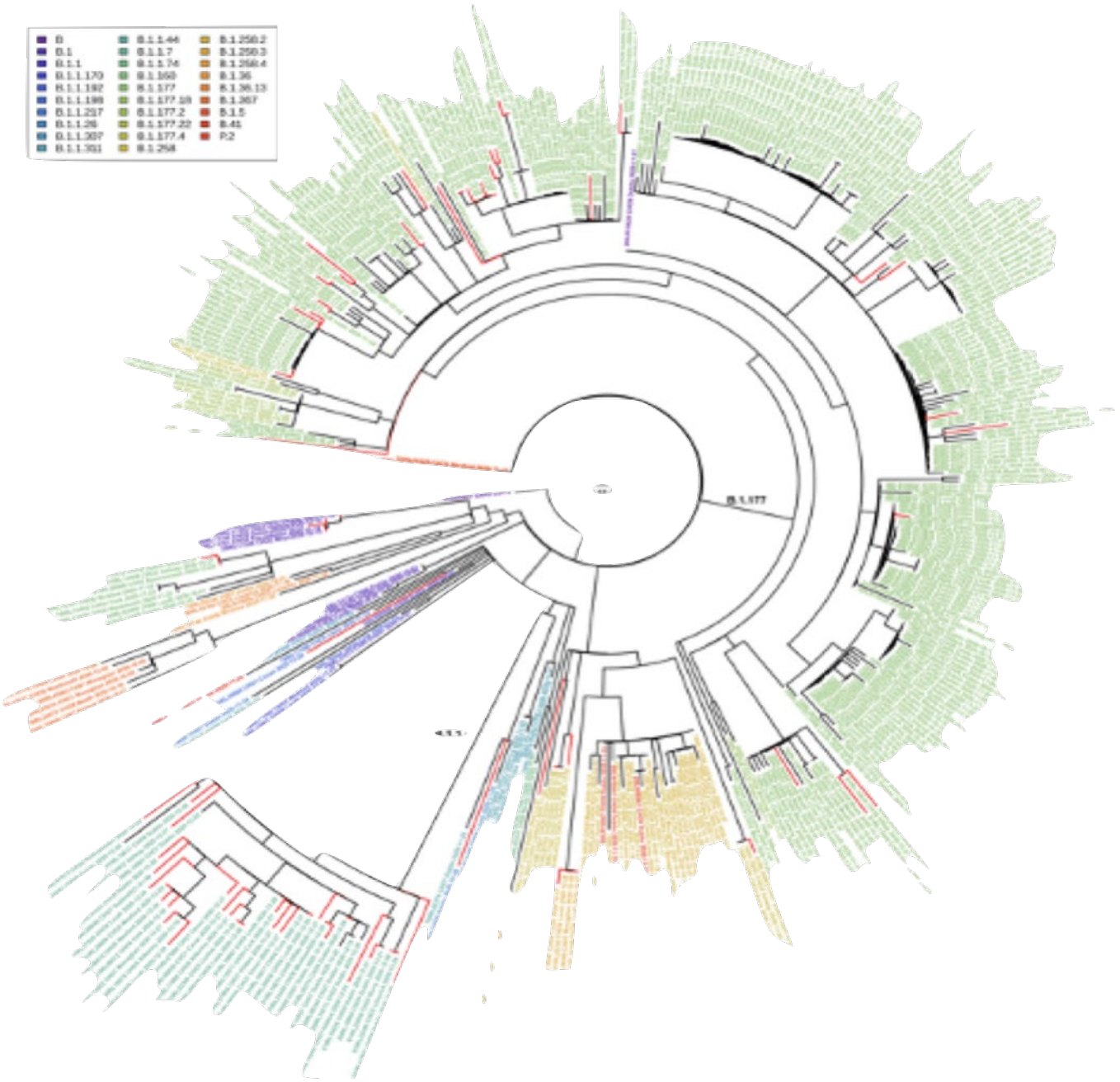


Spent rapid antigen detection test



SARS-CoV-2 identification





Conclusions -

- ***a harmonised protocol for sequencing bacteria and viruses of importance to food safety is established-***
 - **SOPs established and shared for WGS of bacteria and viruses;**
 - **validated protocols now in place;**
 - **harmonised interpretation of risk;**

- ***Established laboratory links between EU and China based teams-***
 - **working relationships in place with CFSA; HKPU;**
 - **collaborations will extend beyond the project lifetime though various joint events planned;**

- **risk assessment – a paradigm shift!**
 - **Demonstrated potential to use big data to assess risk to human health thereby defining a true pathogen?**
 - **future shape of risk assessment based on harmonized genome analysis**



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