



Public Health
England

Salmonella contamination of (imported) fresh edible leaves

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Gastrointestinal Bacteria Reference Service



Top ten Salmonella serovars

63,691 cases of salmonellosis in England 2010-17 where cultures were submitted for characterisation,

41,348 (65%) were due to the top 10 *S.enterica* serovars,

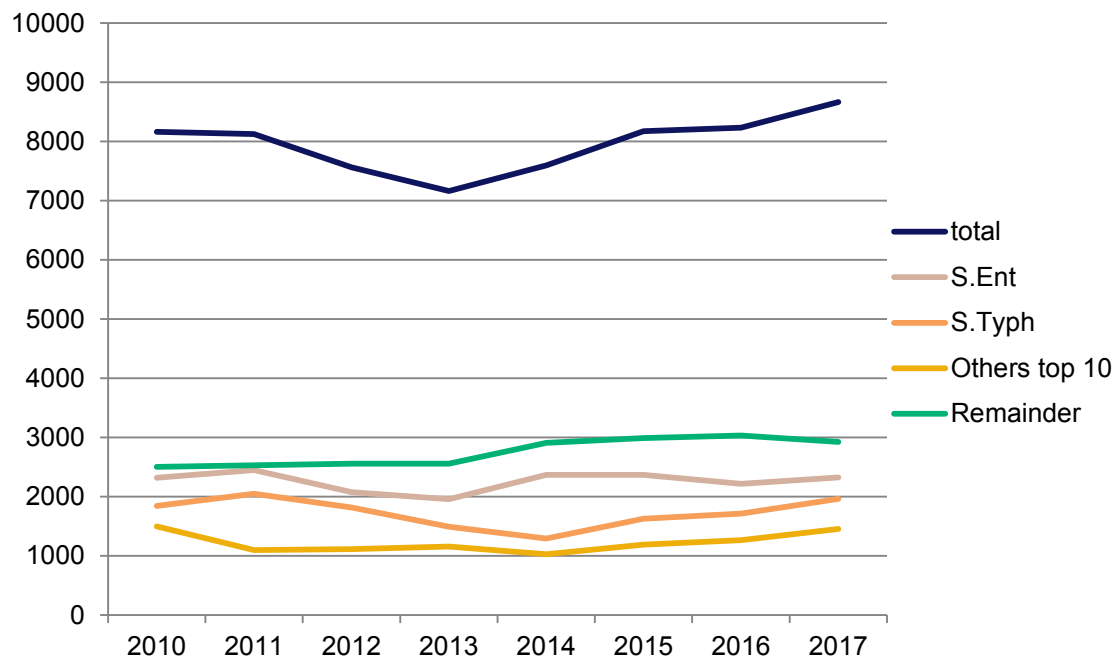
S. Enteritidis, *S.Typhimurium*,

S. Newport, *S.Infantis*,

S.Virchow, *S.Kentucky*,

S. Agona, *S.Stanley*,

S. Java, and *S. Bareilly*.





Cases and outbreaks of salmonellosis

All cases								
Year	2010	2011	2012	2013	2014	2015	2016	2017
Total number of cases	8163	8127	7563	7161	7595	8177	8236	8669
S.Enteritidis	2320	2447	2079	1958	2366	2370	2219	2324
S.Typhimurium	1844	2051	1816	1493	1292	1627	1715	1964
Other top 8 serovars	1496	1099	1112	1155	1029	1192	1266	1456
All other serovars	2503	2530	2556	2555	2908	2988	3036	2925
Numbers of cases in outbreaks (numbers of outbreaks)								
All cases	119 (5)	547 (17)	176 (14)	774 (9)	367 (9)	361 (9)	556 (11)	128 (8)
S.Enteritidis	33 (3)	357 (11)	67 (6)	34 (2)	314 (7)	212 (6)	480 (8)	22 (3)
S.Typhimurium	86 (2)	190 (6)	60 (6)	169 (5)	46 (2)	31 (1)		93 (3)
Other serovars			49 (2)	471 (2)	7 (1)	118 (2)	76 (3)	13 (2)



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How much contamination do we see in imported leaves, are they harbouring antimicrobial resistant genes and are they causing outbreaks?



Betel leaves

Betel (paan/pan) leaves (*Piper betle* L.) traditional product that certain South-eastern Asian populations chew after meals

Imported into the EU particularly to UK and Germany but also to Italy, France, The Netherlands and Portugal





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Betel leaves, initial observations: August 2011

EHO sampled betel leaves as part of an inspection of an ethnic food retailer in Birmingham

Samples tested by FW&E Laboratory in Birmingham

Salmonella enterica serovar Augustenborg isolated

Further sampling at retail and wholesale detected *Salmonella* in 11 out of 64 samples

- 11 (24%) of 45 samples from Bangladesh, 6 *Salmonella* serovars
- 1 (7%) of 15 samples from India

Two consignments tested from Border Inspection Post at Birmingham Airport, both contaminated with *Salmonella*



Salmonella spp. contamination of betel leaves collected at Border Inspection Posts at English airports

Year	Number of samples	
	Total	<i>Salmonella</i> spp. detected (%)
2011	539	198 (37%)
2012	515	120 (23%)
Total	1054	318 (30%)

Collected by Port Health Officers at airports on entry into the EU



Salmonella spp. contamination of betel leaves collected at retail, wholesale and catering

Year	Number of samples	
	Total	<i>Salmonella</i> spp. detected (%)
2011	67	17 (25%)
2012	0	.

Collected by Environmental Health Officers



Assessment in mid-2012

First ~200 isolates identified

- 19% *Salmonella* Virchow
- 10% each *Salmonella* Brunei, *Salmonella* Newport
- Additional 41 serovars of *Salmonella* were identified

A comparison with isolates from betel leaves to those from human cases did not identify betel leaf-consumption attributable morbidity in the UK population



Actions by Risk Managers

2011 after initial isolation of S.Augustenborg, FSA informed who issued RASFFs, discussions held between the EU Commission and the Bangladeshi and Indian High Commissions to ascertain what is being done to reduce contamination in the country of origin

2014 EU Commission implemented a temporary suspension of imported betel leaves originating or consigned by Bangladesh (Decision 2014/88/EU) which was extended in June 2015 (Commission Implementing Decision 2014/510/EU) and then to June 2018 and then to June 2020 (Commission Implementing Decision 2018/935).

2014 Betel leaves from India and Thailand were subjected to an increased level of official control at the designated point of (Commission Implementing Regulation (EU) 323/2014) and this continued for India in September 2015 (Commission Implementing Regulation (EU) 2015/1607).

2016 control of betel leaves from India was extended requiring that consignments be accompanied by results of sampling and analysis by the competent authorities in India for the absence of *Salmonella* (Commission Implementing Regulation (EU) 2016/166).



EU import prohibition for products from Bangladesh, but...

2017,
11 (14%) out of 77
sampled on retail sale
were from Bangladesh,
Salmonella detected in 4 (36%)

Bangladesh betel leaves on
sale in London April 2018





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Salmonella spp. contamination of betel leaves collected at Border Inspection Posts in four English airports

Year	Collected at Border Inspection Posts				Samples collected at retail, wholesale or catering	
	Number of samples		Number of consignments			
	Total	Salmonella spp. detected (%)	Total	Salmonella spp. detected (%)	Total	Salmonella spp. detected (%)
2011	539	198 (37%)	108	83 (77%)	67	17 (25%)
2012	515	120 (23%)	103	62 (60%)	0	.
2013	481	78 (16%)	97	42 (43%)	11	1 (9%)
2014	145	16 (11%)	24	8 (33%)	42	1 (2%)
2015	116	24 (21%)	19	9 (47%)	22	3 (14%)
2016	78	19 (24%)	21	10 (77%)	1	0
2017	16	0	6	0	77	11 (14%)
Total	1890	455 (24%)	378	214 (57%)	220	33 (15%)



Salmonella spp. contamination of betel leaves collected at Border Inspection Posts

Year	Number of samples		Number of consignments	
	Total	<i>Salmonella</i> spp. detected (%)	Total	<i>Salmonella</i> spp. detected (%)
Bangladesh	1064	278 (26%)	207	133 (64%)
India	415	70 (17%)	82	38 (46%)
Malaysia	65	17 (26%)	17	8 (47%)
Nepal	5	1 (20%)	1	1 (100%)
Sri Lanka	23	2 (9%)	6	2 (33%)
Thailand	135	23 (17%)	30	10 (38%)
Not known	183	64 (35%)	35	60 (63%)



Characterisation *Salmonella* from Border Inspection Posts

Where multiple cultures from the same sample or consignment were characterised, only one representative isolate per serovar per consignment has been included

332 representative isolates

44 *S. enterica* subspecies I serovars identified,

plus un-named, *Salmonella di arizonae*, *Salmonella salamae*

- 17% *Salmonella* Virchow
- 10% *Salmonella* Java
- 8% *Salmonella enterica* (un-named)
- 7% *Salmonella* Brunei
- 7% *Salmonella* Litchfield
- 6% *Salmonella* Newport



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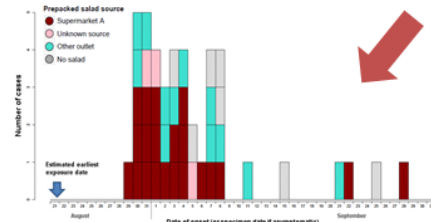
WGS Typing and antimicrobial resistance of Salmonella from leaves



WGS Overview

Cluster 15:2378
1 isolates within search criteria, 3 isolates in the database

SNP Address	Motis ID	Ext ID	Sending Lab	PHE Region	PHE Centre	HPT	Country	Sample/Isolate Type	Info	NHS Number	First Name	Surname	Date of Birth	Sex	Post Code	Receipt Date	Sample Date	Report Date	Foreign Travel	Organism Identified
2.154.315.2041.2320.2378.3088	H160280263	16.0900451	DX6780100-HPA WEST MIDLANDS	Midlands and East of England	West Midlands	West Midlands East	England	Human Faeces								2016-01-15	2016-01-07	2016-01-19	Morocco	Escherichia coli
2.154.315.2041.2320.2378.2814	H153980184	15-2077330-1	DX6120200-HPA S.WEST-BRISTOL	South of England	South West	Avon, Gloucestershire and Wiltshire	England	Human Faeces								2015-09-25	2015-09-18	2015-10-06	Morocco	Escherichia coli
2.154.315.2041.2320.2378.2546	H152920519	M.15.1798871	WHISTON HOSPITAL L35	North of England	North West	Cheshire and Merseyside	England	Human Faeces								2015-07-14	2015-07-09	2015-07-16	Morocco	Escherichia coli



Sub-culture and extraction

1 - 2 days

Sequencing

4 - 7 days

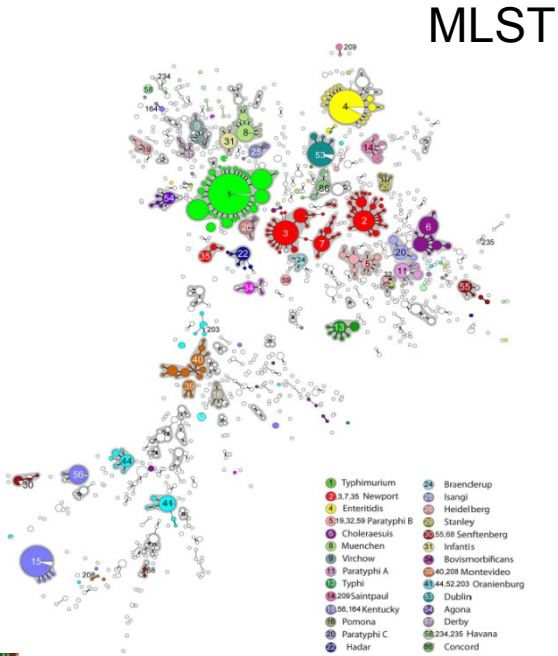
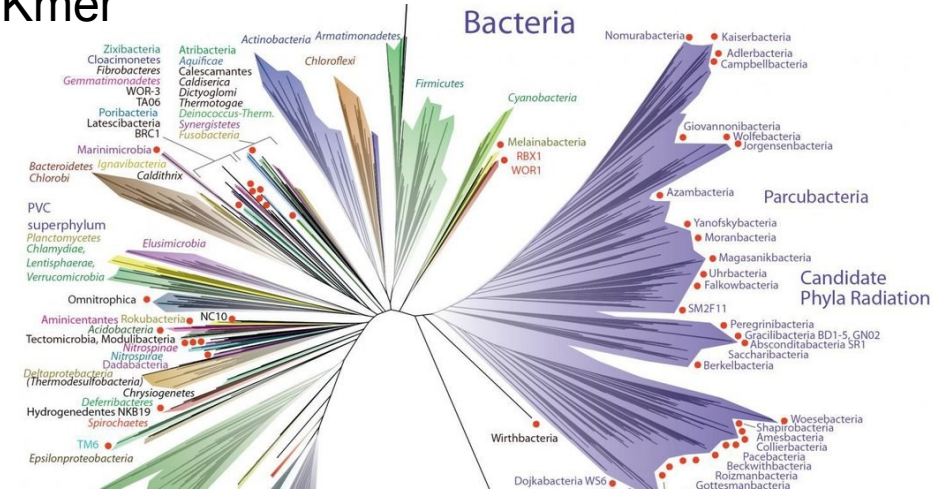
Bioinformatics pipeline

<1 day

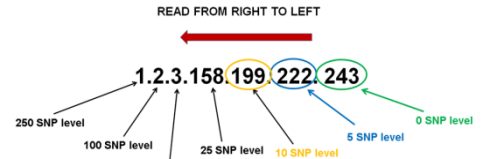
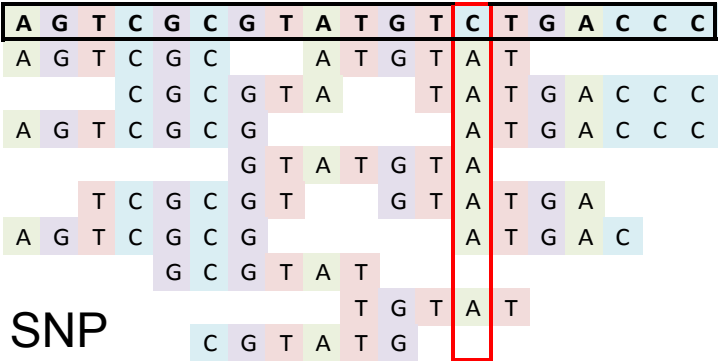
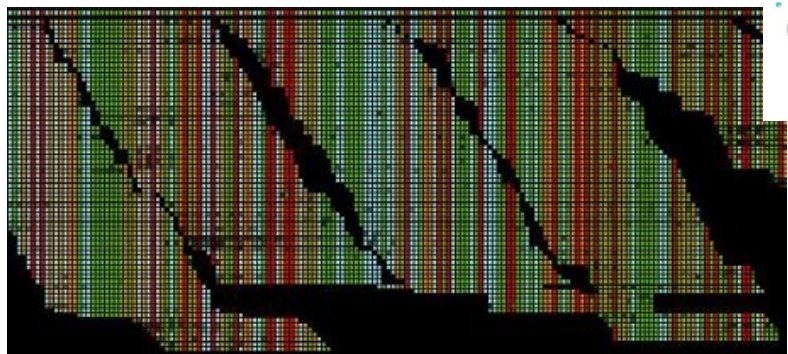
Technical and Medical Validation
<1 day for typical profiles

SNP address on GDW

Atypical profiles or rare subgroups may require additional work for validation purposes.

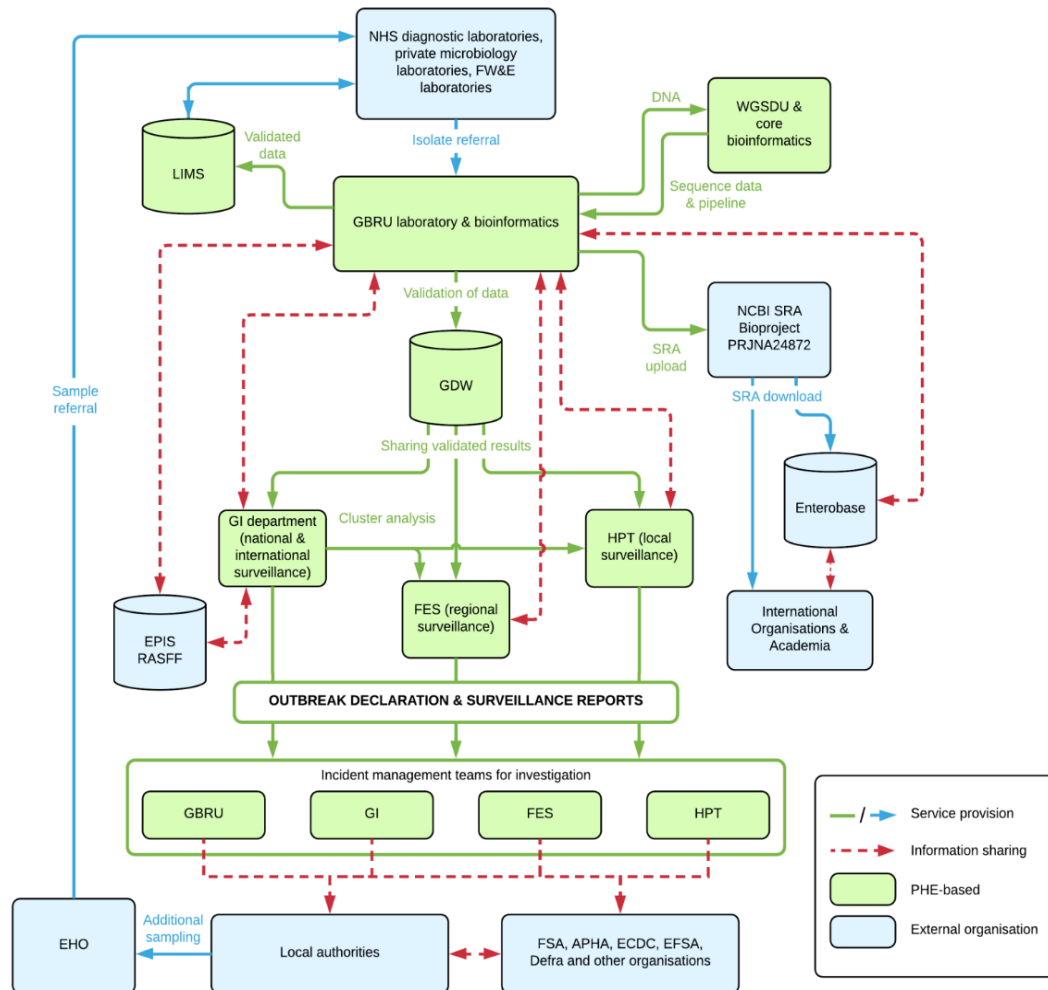


Illumina Short Reads



Phylogeny
SNP Address

Overview of service provision and information sharing of WGS in UK





Submission of *Salmonella*

Salmonella detected in 488 samples from leaves since 2011

At least one *Salmonella* culture was submitted to GBRU from 475 (97%) of the samples

- 420 (89%), conventional serotyping (2011-13)
- 55 (11%) whole genome sequencing since 2014

No outbreaks linked or putatively linked to betel leaves were detected

Isolates from two sporadic human cases matching at ≤ 5 SNP level with betel leaf isolates



Salmonella

All 44 *S. enterica* isolates analysed and SNPs established
>5 SNPs differences with isolated from two human cases were identified, both in 2017:

1 *S. Newport* and *S. Bareilly* (betel leaf from Bangladesh)

Betel leaves (n = 11)

5	Salmonella Newport
3	Salmonella Bareilly
1	Salmonella Hvittingfoss
1	Salmonella Lichfield
1	Salmonella Typhimurium

Curry leaves (n = 31)

1	Salmonella Aberdeen
1	Salmonella Arizonae
1	Salmonella Bareilly
1	Salmonella Cerro
3	Salmonella Fulica
1	Salmonella Guildford
2	Salmonella Hvittingfoss
1	Salmonella Javiana
1	Salmonella Kasenyi
1	Salmonella Kentucky
1	Salmonella Morehead
1	Salmonella Mount-pleasant
1	Salmonella Newport
2	Salmonella Rubislaw
3	Salmonella Stanleyville
2	Salmonella Typhimurium
1	Salmonella Umbilo
4	Salmonella Uzaramo
3	Salmonella Weltevreden

Banana leaves (n = 1)

1	Salmonella Virchow
Other leaves (n = 1)	
1	Salmonella Poona



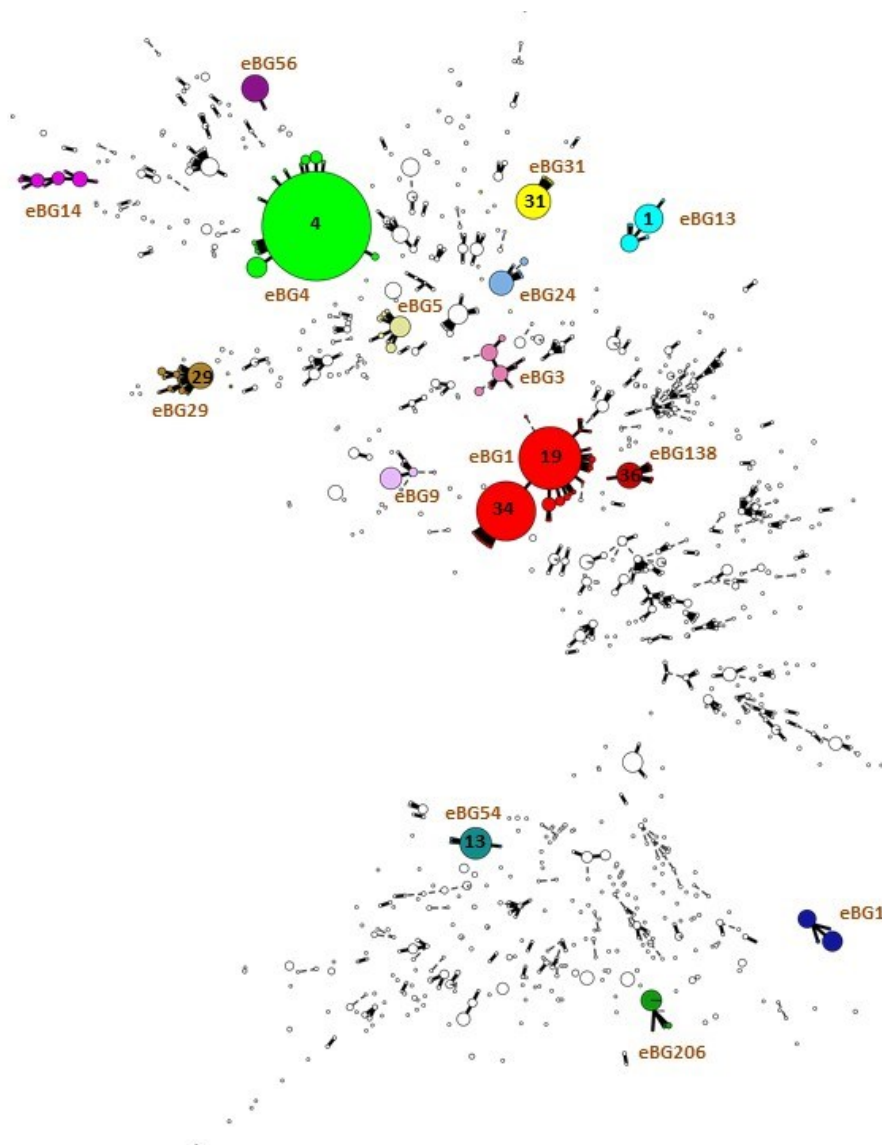
Two sporadic human cases

Patient 1. 74 year-old female of Asian origin in the North West

- S.Bareilly (ST 203) isolated from the faeces in August 2015
- S.Bareilly (ST 203) matched at the 0-SNP level to an isolate from betel leaves , country of origin not known
- Sampled in June 2015 from a retail establishment from the same region <18 miles from patient's home address

Patient 2. 44 year old male of East Asian origin in

- S.Newport (ST 31) isolated from the faeces in October 2017
- S.Newport (ST 31) matched at the <5-SNP level to an isolate from betel leaves from Bangladesh
- Betel leaves collected from retail sale in July 2017 from a different region to the patient's home address



Colour	eBG	Serovar	Number
Green	4	Enteritidis	4866
Red	1	Typhimurium	3025
Yellow	31	Infantis	469
Cyan	13	Typhi	445
Teal	54	Agona	387
Brown	29	Stanley	330
Purple	56	Kentucky	276
Dark Blue	11	Paratyphi A	274
Dark Red	138	Typhimurium	263
Light Blue	24	Braenderup/ Larochele	249
Pink	3	Newport	244
Light Green	5	Paratyphi B/ Java	239
Magenta	14	Saintpaul	226
Light Purple	9	Virchow	216
Dark Green	206	Bareilly/Richmond	176

Conclusions

The high discriminatory power of WGS provides greater confidence about a highly heterogeneous population structure - see figure

Isolates from every individual consignment is different

There appears to be a low rate of transmission – due to heterogenous serovars and under reporting of mild illness?



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Detecting outbreaks using Enterobase

Curry leaves



Banana leaves



Enterobase

Salmonella

Species Home | Data | View | Workspace

Tasks | Upload Reads | Search Strains | Find ST(s) | Curate Strains | Show My Jobs | My Buddies | Locus Search | Download Schemes

Uberstrain

MLST Query

Scheme: cgMLST V2 + HierCC

☐ Search On Allele

☒ Search On ST

ST: 3399 Max Number Mismatches: 20

Cancel Submit

Pandan leaves



Betel leaves



Results - Transmission



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Leaf type	Serovar	Country of origin	Date	Allele (ST) number	Enterobase results:			
					Source	Country	Date	Allele difference
Betel	Newport	Bangladesh	13/06/2017	106938	Human	UK	04/10/2017	1
Betel	Bareilly	Unknown	17/07/2015	119886	Human	UK	01/06/2015	12
Curry	Cerro	Unknown	09/11/2017	3399	Human	UK	18/11/2017	1
Curry	Stanleyville	Unknown	09/08/2017	106975	Human	UK	26/08/2017	0
Curry	Newport	Kenya	11/02/2014	27995	Human (n=2)	UK	2014	10, 11
					Human (n=4)	UK	2015	9, 10, 13
					Human	UK	2016	6
					Human (n=9)	UK	2017	9 to 14
					Human (n=2)	UK	2018	8, 12
					Human	Ireland	2017	9
					Food	Uganda	2014	13
					Poultry	Uganda	2014	8
					Unknown	Kenya	2012	9
					Unknown	Unknown	2017	12
Curry	Agona	Pakistan	06/03/2013	21224	Human (n=9)	UK	2013	0 to 3
					Human (n=2)	UK	2014	4, 9
					Human (n=5)	UK	2015	6 to 10
					Human (n=5)	UK	2016	4 to 15
					Human	Canada	2016	2
					Human	Australia	2017	5
					Human	US	2015	11
					Unknown	Ethiopia	2017	10

Results - Antibiotic resistance



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- Müller-Hinton agar dilution method and WGS (genefinder)
- Leaf isolates

Leaf type	Serovar	No. of isolates	Country of origin	Resistant to:									% MDR isolates
				AMP	AZM	CHL	NAL	SMX	TCY	TMP	AMX	SXT	
Curry	Agona	1	Pakistan					1	1	1		1	100
Curry	Weltevreden	1	Unknown	1	1	1		1	1	1	1	1	100
Curry	Weltevreden	1	India				1						0

Antibiotic class	Resistance gene	Number
Chloramphenicol	catA-2[v]	1
Fluoroquinolones	qnrS-1	1
	gyrA_SET[87:D-N]	1
Macrolides	mph-(A)	1
Trimethoprim	dfrA-5	1
	dfrA-12	1
Tetracyclines	tet(A)-1[v]	2
	tet(M)[v]	1
Sulphonamides	sul-1[v]	2
	sul-3	1

AMP, ampicillin; AZM, azithromycin, CHL, chloramphenicol; NAL, nalidixic acid; SMX, sulfamethoxazole; TCY, tetracycline; TMP, trimethoprim; AMX, amoxicillin; SXT, trimethoprim-sulfamethoxazole

Conclusion

- Eight (26.7%) out of 30 serovars from imported leaves and were in the top 10 NTS serovars reported in England and Wales in 2016
- Three (2.6%) leaf isolates were resistant, two (1.7%) MDR. No genotypic or phenotypic resistance to ertapenem or colistin
- Eight (7%) of the leaf isolates were associated with clinical cases and other possible niches
- **Importance:** due to international trade and the mass distribution of food, AMR *Salmonella* and *Salmonella* serovars considered rare can be imported into the UK and be associated with clinical cases or outbreaks



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Are we seeing outbreaks from imported produce such as leaves, herbs, spices?

*Atypical and rare outbreak of Salmonella
Adjame*

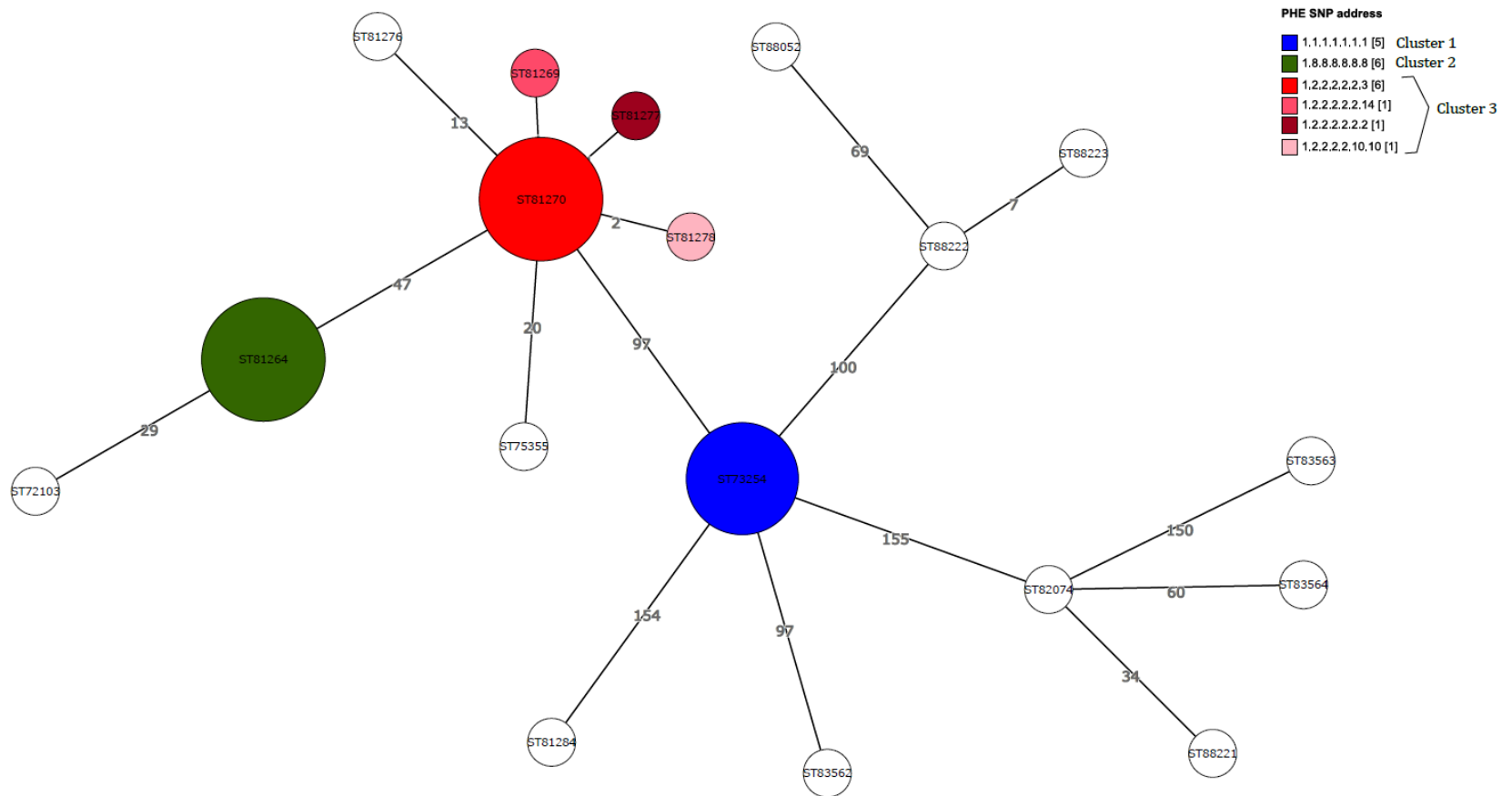
SNP address: Heterogenous

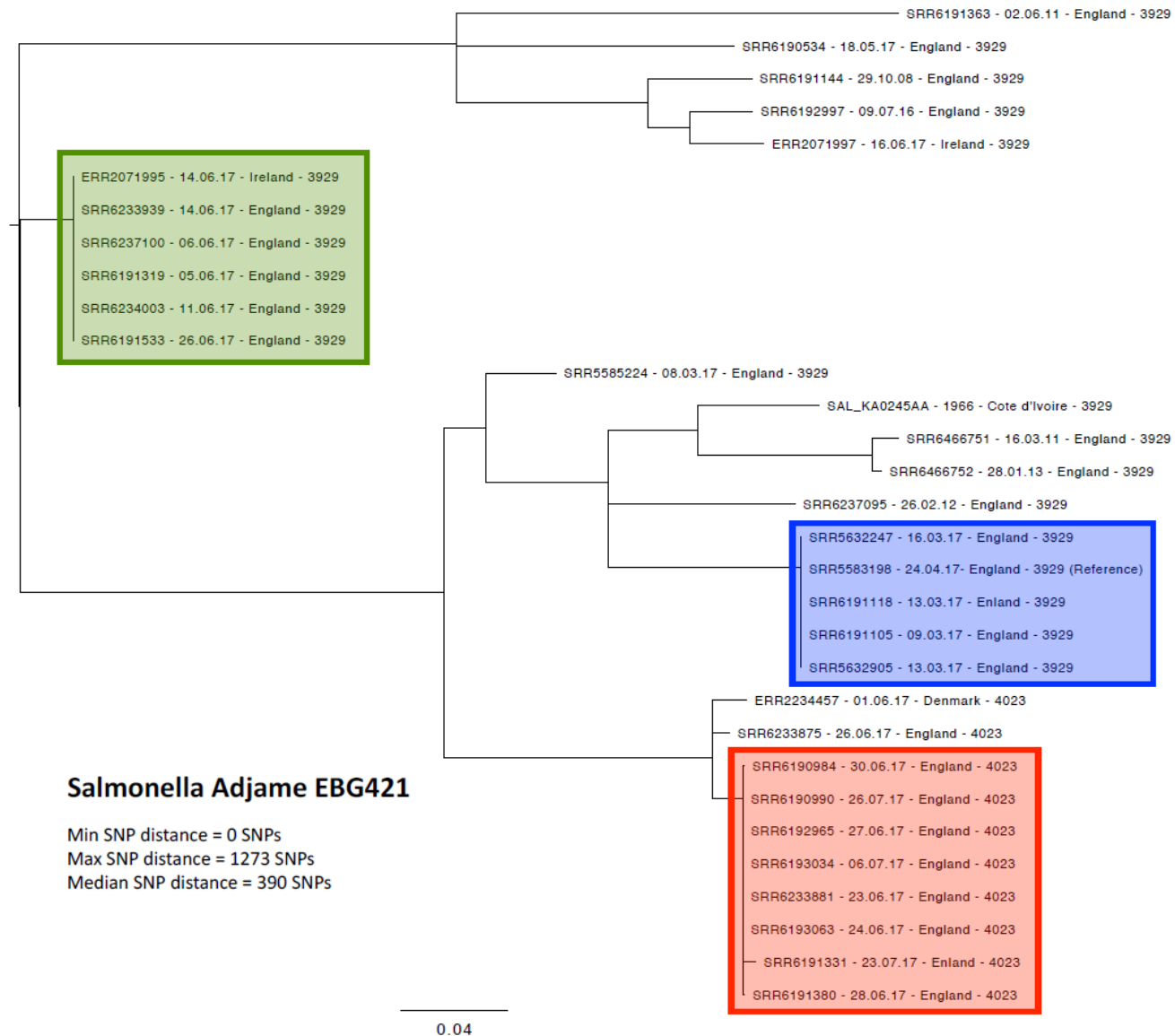


Outbreak background

- In August 2017, an increase in the number of *S. Adjame* isolates was reported initially
- An outbreak case was defined as a person resident in England with a clinical sample from 01 June 2017 to 27 July 2017 from which *S. Adjame* was isolated.
- Trawling questionnaires were completed looking at food exposures
- An (Epidemic Intelligence Information System) EPIS urgent enquiry was posted to ascertain if there had been any *S. Adjame* cases recently in Europe
- SNP typing was not initially available and GBRU performed cgMLST via the publically available database - Enterobase.

cgMLST analysis showed a large diversity of strain
with 3 main clusters







Conclusions

- This was the first reported outbreak of *S. Adjame* described since its isolation in 1966
- While the cases of *S. Adjame* were linked epidemiological in time/person/place, WGS showed marked heterogeneity indicating a multi-strain outbreak, atypical of *Salmonella enterica* outbreaks PHE usually investigates
- Although imported produce, most likely leaves, herbs or spices were suspected vehicles, there was only descriptive epidemiological evidence supporting this. Handful of European cases
- it is likely that the vehicle of infection was imported from an area outside the EU where the strain is persistently present in a source environment or host population where strains have had the time to diversify and sources such as sewage would contain multiple genetically diverse strains.
- The interpretation of genomic analysis in regards to *Salmonella enterica* outbreak investigations needs to be adaptable to the particular circumstances of the outbreak in terms of case definition.
- cgMLST was comparable to SNP for cluster detection in this outbreak and is a potential method for outbreak comparisons but further validation is required.

Large outbreak of multiple gastrointestinal pathogens associated with fresh curry leaves in North East England, 2013.

Waldram A¹, Lawler J², Jenkins C³, Collins J⁴, Payne M⁴, Aird H⁵, Swindlehurst M⁵, Adak GK⁶, Grant K³, Ready D³, Gorton R¹, Foster K².

Author information

Abstract

A total of 592 people reported gastrointestinal illness following attendance East England in February/March 2013. Epidemiological, microbiological and source and prevent further cases. Several epidemiological analyses were re-capture to estimate the true burden of cases. Indistinguishable isolates on fresh curry leaves used in one of the accompaniments served at the event and Shigella also contributed to the burden of illness. Analytical studies for particular stall and with food items including coconut chutney which contain chain and food preparation techniques identified a lack of clear instruction uncertainty about their status as a ready-to-eat product. We describe the outbreak in England, involving several gastrointestinal pathogens including a strain of:

Further Reading

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Public health risks associated with *Salmonella* contamination of imported edible betel leaves: Analysis of results from England, 2011–2017

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MICROBIAL GENOMICS

OUTBREAK REPORT

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Genomic approaches used to investigate an atypical outbreak of *Salmonella* Adjame

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Abstract

In 2017, an outbreak of gastroenteritis in England attributed to *Salmonella* Adjame was detected and investigated. With the introduction of whole genome sequencing (WGS) for microbial typing, methods for comparing international outbreak data require evaluation. A case was defined as a person resident in England with a clinical sample from 1 June 2017 to 27 July 2017 from whom *S. Adjame* was isolated. Cases were interviewed and exposures analysed. Backward tracing of food provenance was undertaken. WGS was performed on isolates from cases and historical isolates and compared using Public Health England's SnapperDB high-quality SNP pipeline and Enterobase's *Salmonella* core genome multi-locus sequence typing (cgMLST) scheme. In total, 14 cases were identified. The majority were vegetarian, probably of South Asian descent, with a median age of 66.5 years with no recent international travel reported. Cases consumed a range of fresh food products including bakes and salads bought from South Asian sources. Backward tracing did not identify a common source. WGS

ORIGINAL ARTICLE

Imported edible leaves collected at retail sale in England during 2017 with an emphasis on betel and curry leaves: microbiological quality with respect to *Salmonella*, Shiga-toxin-producing *E. coli* (STEC) and levels of *Escherichia coli*

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