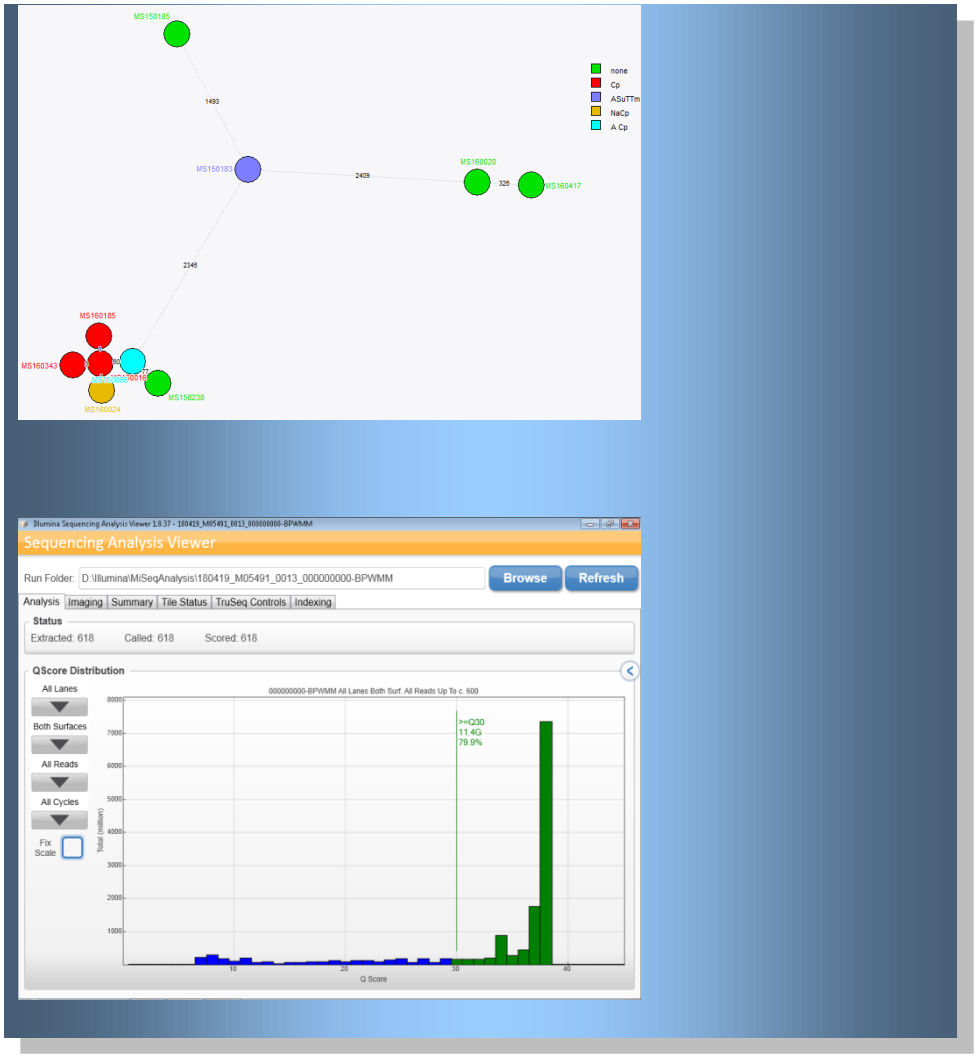
NATIONAL *SALMONELLA, SHIGELLA & LISTERIA* REFERENCE LABORATORY OF IRELAND (HUMAN HEALTH)



ANNUAL REPORT FOR 2018

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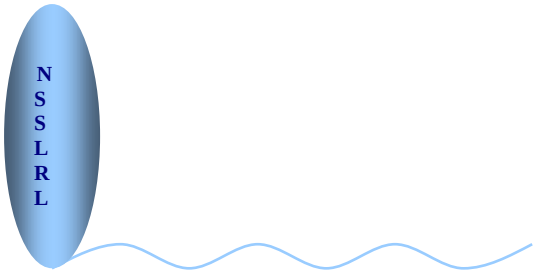

Introduction

The National *Salmonella*, *Shigella* & *Listeria* Reference Laboratory was established in 2000 with support from the Department of Health and Children to provide reference services related to human health. It is a public service laboratory which currently operates with 3 WTE scientific staff. The NSSLRL website is at http://www.saolta.ie/publications

The reference laboratory now uses predominantly molecular methods based on genome sequencing to characterise isolates. WGS was performed on all isolates of *Salmonella*, *Shigella* and *Listeria monocytogenes* received in 2018. This process can be considered as fingerprinting of these bacteria. The goal of fingerprinting is to assist relevant agencies in protecting public health by identifying and interrupting chains of transmission.

The Laboratory is committed to providing a high quality and timely service and has achieved accreditation to the ISO15189[1] standard from the Irish National Accreditation Board (INAB). The continued success of the laboratory is entirely dependent on the support of the staff in the laboratories that submit isolates for typing. My colleagues and I appreciate that the preparation, packing and dispatch of isolates is a significant burden and would like to thank you for your support over the years. We acknowledge also the ongoing support of the Information and Communication Technology services in supporting the ICT demands related to WGS.

I would also like to acknowledge the support of all those agencies with whom we work closely to ensure that the service we provide works as information for action. In particular I would like to thank Galway University Hospitals, NUI Galway, the Food Safety Authority of Ireland, the Health Protection Surveillance Centre and colleagues in Public Health Departments and Environmental Health Departments throughout the country and to acknowledge the work of colleagues in the National Reference Laboratory *Salmonella* (Food, Feed and Animal Health)[1].

Summary Points.

Salmonella spp.

A total of 366 *Salmonella* isolates from 362 patients were typed. This represents a 6.7% decrease in the number of isolates received compared with 2017. *S*.Typhimurium and its monophasic variant together accounted for 30.1% of all cases and *S*.Enteritidis accounted for 25.7%. *S*.Typhi (typhoid fever) was detected from 11 patients and *S*.Paratyphi A (paratyphoid fever) was detected from, 5 patients. A history of recent travel was recorded for 15 of the 16 patients with all but one of these reporting travel to the Indian subcontinent. Multi-drug resistance (resistant to three or more different classes of antibiotics) was detected in 21.7% of isolates.

Listeria monocytogenes.

The NSSLRL received 18 *Listeria monocytogenes* isolates from human clinical samples in 2018 compared with 11 in 2017. This is likely to be in part related to improved submission of isolates by clinical laboratories in 2018. There were 16 from blood cultures, 1 from CSF and 1 from a placental swab and 11 isolates were serotype 4b. Whole genome sequencing did not suggest a common source of infection for any of the 18 cases (no clustering detected in 2018).

Shigella spp.

Shigella isolates were typed from 75 patients in 2018. These consisted of 48 *S.sonnei* , 25 *S.flexneri*, and 2 *S.boydii*. The majority of isolates, 83.3 % were multi-drug resistant (resistance to three or more different classes of antibiotics) of which 8 were ESBL producers. Shigella infection in Ireland is associated with sexual transmission in men who have sex with men.

If you have any comments or questions arising from the report please feel free to contact me at the email address given below.

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[1] ISO 15189:2012 Medical laboratories -- Requirements for quality and competence

Salmonella

In 2018, 449 isolates from both clinical and food sources were submitted to the National *Salmonella, Shigella* & *Listeria* Reference Laboratory for *Salmonella* typing. When non-*Salmonella*, QC, contaminants and duplicate isolates were removed a total of 366 *Salmonella* isolates were typed. This represents a 6.7% decrease in the number of isolates received compared with 2017.

There were 362 human clinical isolates, including 326 faecal isolates, 25 from blood (including 9 *S*.Typhi and 4 *S*.Paratyphi A), 4 other invasive isolates, and 7 urine isolates. *S*.Typhimurium (n = 56) and its monophasic variant 4,[5],12:i:- (n = 53) and *S*.Enteritidis (n = 93) predominated (Table 2). There was marked seasonal variation with the highest number of isolates occurring in months August to November. This coincides with the warmer months of the year and with the peak season for foreign travel (Fig.1) and may be related in part to one or both of these factors.

In some cases more than one isolate was received from a patient. For example we may have received an invasive isolate (for example. from a blood culture) and an isolate from faeces from the same patient. Where invasive and faecal isolates come from the same patient, only the invasive isolate is recorded to avoid duplication. The average turnaround time for reports on human isolates was 9 days (range 2-34 days). The number of human *Salmonella* isolates received reached its lowest point in 2014 (n=258) but the annual numbers increased progressively through to 2017. Although there was a small decline in 2018 the number of cases in 2018 was 40% higher than observed in 2014.

Table 1: Number of *Salmonella* isolates received in NSSLRL

Year	Human	Non-human
2018	362	4
2017	388	5
2016	309	68
2015	286	257
2014	258	261
2013	345	312
2012	319	391
2011	320	381
2010	364	559
2009	364	368
2008	447	815
2007	457	653

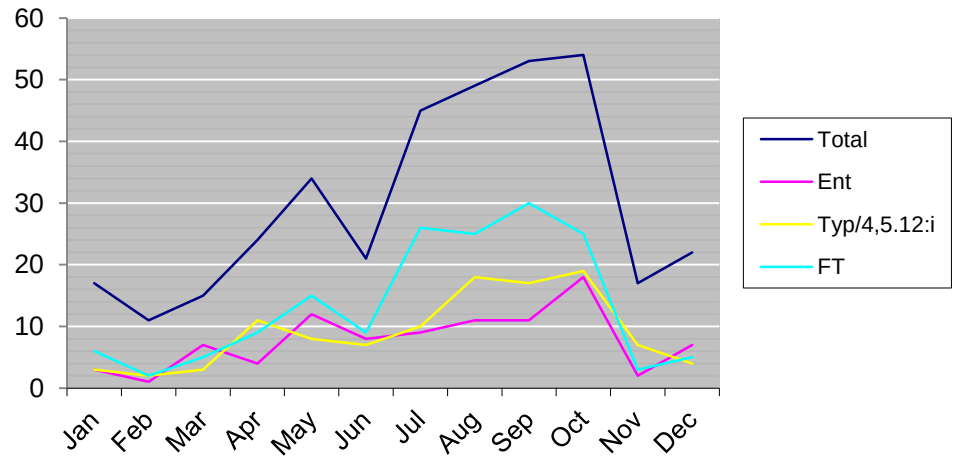
2006	430	308
2005	357	494
2004	420	650
2003	486	634
2002	394	540
2001	508	574
2000	636	21

Table 2: Top 21 serotypes of Human Isolates (inc typhoidal)

Serotype	Frequency	%
Enteritidis	93	25.7
Typhimurium	56	15.5
Monophasic Typhimurium[1]	53	14.6
Newport	18	5.0
Typhi	11	3.0
Agona	9	2.5
Kentucky	8	2.2
Infantis	7	1.9
Braenderup	6	1.7
Stanley	6	1.7
Virchow	6	1.7
Java	5	1.4
Paratyphi A	5	1.4
Bredeney	4	1.1
Agama	3	0.8
Brandenburg	3	0.8
Bovismorbificans	3	0.8
Coeln	3	0.8
Derby	3	0.8
Litchfield	3	0.8
Rissen	3	0.8
Others	54	14.9
Total	363	100

[1] The antigenic formula 4,[5],12:i:- is that of *S*. Typhimurium except that the phase 2 antigen is not expressed. These isolates are generally referred to as monophasic *S*. Typhimurium and are so called in this report.

Figure 1 Seasonal Variation in numbers of *Salmonella* isolates [1]



[1.] The line "FT (Foreign Travel)" describes the number of cases of salmonellosis for which an association with recent foreign travel was reported to NSSLRL. Reporting of recent foreign travel is likely to be incomplete.

It is important to note that there is always an interval gap between the time of onset of symptoms and date of isolate receipt in the NSSLRL. This includes time taken for patient to access doctor, taking and transporting the sample to the primary laboratory, isolation of *Salmonella*, and referral to NSSLRL. Fig 1 refers to date of receipt in the NSSLRL.

Salmonellosis non-typhoidal

S.Typhimurium and its monophasic variant.

S.Typhimurium 4,[5],12;i:2 and its monophasic variant (4,[5],12;i:-) together accounted for 30.1% of all cases of *Salmonella*.

S.Enteritidis

S.Enteritidis accounted for 25.7% of all cases of *Salmonella*.

Salmonellosis Typhi and Paratyphi

Eleven isolates of *S*.Typhi and 5 of *S*.Paratyphi A were received. A history of recent travel was recorded for 10 of the *S*.Typhi isolates; all to Asia and 9 to the Indian subcontinent. The *S*.Paratyphi A isolates all had a reported history of recent travel to India.

Antimicrobial resistance

Susceptibility testing was not performed on isolates received after 1st October 2018 (apart from MS180365 =ASuNaCpCol), MS180371 (none) and MS190393 (none)) so 273/363 were tested.

This was because from that date, following extensive validation, antimicrobial susceptibility patterns were predicted from the whole genome sequence thus reducing analytical costs and laboratory waste.

More than half isolates (146 of 272, 53.7%) were susceptible to all antimicrobial agents tested. Twenty one point seven percent of isolates (n = 59) were multi-drug resistant (three or more different classes of antibiotics). Thirty two point two percent of isolates that were multi-drug resistant, 32.2% (n = 19) had the profile of resistance to ampicillin, sulphonamide and tetracycline (ASuT) and were mainly monophasic S. Typhimurium.

One extended spectrum beta-lactamase (ESBL) producing isolate was detected. Seventy isolates of *Salmonella* resistant to ciprofloxacin were detected (25.7%).

High level resistance to ciprofloxacin (>2mg/l) is rare among *Salmonella* but a ciprofloxacin-resistant *S.* Kentucky clonal group has arisen and spread from North Africa in the last decade. Seven such isolates were typed in the NSSLRL in 2018, 1 of which had a history of foreign travel to India, another to Morocco, another to Indonesia and another with foreign travel but country not stated.

The NSSLRL added two new antibiotics, azithromycin and tigecycline, to its testing panel at the end of 2013, based on advice from the European Centre for Disease Prevention and Control (ECDC), to detect emerging resistances. Resistance to azithromycin was detected in isolates from 3 patients including a *S*.Brandenburg, a *S*.Choleraesuis with foreign travel to Colombia and a *S*.Enteritidis with travel to Poland. No isolates exhibited tigecycline resistance.

Travel related infection

A history of recent foreign travel was recorded in 160 of the 362 (44.2%) human cases of infection (Table 3). Ireland was noted as the country of infection in 26.2 % (n = 95) of cases while 29.6 % (n = 107) had no country of infection recorded. Spain, Bosnia (isolates from Bosnia related to one outbreak), Thailand and India were the most commonly recorded travel destinations. *S*. Enteritidis accounted for a high proportion of isolates associated with travel to Spain (50%). Forty of 93 (43 %) *S*.Enteritidis isolates were associated with foreign travel compared to 34.9 % for *S*. Typhimurium and its monophasic variant combined. Although NSSLRL does not have access to data on the number of Irish people who travel to each country it is likely that the number of cases associated with each country is at least in part accounted for by the popularity of the country as a destination.

Table 3: Foreign travel history for *Salmonella* isolates

Continent	Country	Number
Europe (n = 72)		
	Spain	24
	Bosnia	16
	Poland	7
	Turkey	4
	United Kingdom	4
	Bulgaria	2
	Hungary	2
	Malta	2
	Ukraine	2
	France	1
	Germany	1
	Italy	1
	Lithuania	1
	Moldova	1
	Portugal	1
	Portugal & Spain	1
	Russia	1
	The Netherlands	1
Africa (n = 16)		
	Morocco	3
	Nigeria	2
	South Africa	2
	Ethiopia	1
	Ghana	1
	Kenya	1
	Mauritius	1
	Somalia	1
	Sudan	1
	Tanzania	1
	Tanzania & Spain	1
	The Congo	1

Australasia (n = 62)		
	Thailand	14
	India	13
	The Philippines	7
	Pakistan	6
	Vietnam	6
	Indonesia	5
	Cambodia	2
	Nepal	2
	Afghanistan	1
	Bangladesh	1
	Mongolia	1
	Singapore	1
	Sri Lanka	1
	U.A.E.	1
	Asia*	1
Americas (n = 8)		
	Cuba	3
	Brazil	1
	Columbia	1
	Mexico	1
	Trinidad	1
	USA	1
Foreign travel with country unknown		2

* Country not stated

Clusters

Forty two clusters of cases involving 119 isolates were identified in 2018. *S*. Typhimurium/monophasic S. Typhimurium was involved in 13 clusters (46 isolates) while *S*. Enteritidis was implicated in 16 clusters (42 isolates).

Five of the clusters (11 patients) were family outbreaks, that is all patients affected were from one family.

The NSSLRL liaises with the European Centre for Disease Control (ECDC) in the investigation of clusters and outbreaks that may have an international dimension.

Animal Contact

A history of animal contact was recorded for 68 patients with salmonellosis including contact with reptiles, birds, fish, horses, dogs and farm animals. Dogs were the most common contact animal (n = 36) while contact with cats was less common (n = 13).

Links included patients with *S*. IV 50:g,z51, *S*. IIIa 13,23:z4,z23:- and S. Bochum associated with reptiles and people working with food animals with *S*.Agama, S.4,[5],12:i:- and *S*.Dublin.

In total 18 isolates of *Salmonella* (approx. 5 % of all human cases) were associated with contact with exotic animals although in these animals may not have been the source of the infections. Public information on the risk (particularly to children) of contact with reptiles has been circulated http://www.hpsc.ie/a-z/zoonotic/reptilesandrisksofinfectiousdiseases/

Many of the patients that had a history of animal contact also had other risk factors, for example. recent history of foreign travel or consumption of particular food products. It is important to note that *Salmonella* is primarily a foodborne disease and that contact with animals such as dogs and cats is very common in the general population therefore contact with an animal species should not be taken to indicate that the animal is the likely source of infection.

Non-Human isolates

The NSSLRL only performs full characterisation on non-human isolates from official food laboratories. In 2018, 4 isolates of *Salmonella* of non-human origin were submitted to the NSSLRL. Two of the isolates were from poultry, 1 was from a herb while the *S*.Enteritidis isolates was from a food sample associated with an outbreak.

Table 4 Serotypes among non-human isolates

Serotype	Frequency	%*
Derby	1	25
Freetown	1	25
Enteritidis	1	25
Montevideo	1	25
Total	4	100%

Antimicrobial Resistance among non-Human isolates

Antimicrobial susceptibility testing was not performed on the 4 non-human isolates.

Laboratory Contamination

False-positive *Salmonella* results due to laboratory cross-contamination are a serious problem for laboratories and can be difficult to detect. Cross contamination in a laboratory can result in inappropriate diagnosis of patient infection or in unfounded concerns regarding the safety of a food product. Detailed subtyping of isolates by the NSSLRL helps in detection and confirmation of laboratory contamination incidents (Role of Subtyping in Detecting *Salmonella* Cross Contamination in the Laboratory; BMC Microbiology: 9; 155).

We would like to reiterate our request that all laboratories involved in testing *Salmonella* from any source use *Salmonella* Nottingham NCTC 7382 as their positive control.

The use of direct PCR based detection systems for enteric pathogens may lead to less incidents of cross contamination compared to conventional methods, especially the use of liquid selection methods such as Selenite broth. This is because broth cultures yield very high concentrations of bacteria and the liquid give potential for splash contamination.

Listeria monocytogenes

The NSSLRL received 18 *Listeria monocytogenes* isolates from human clinical samples in 2018. These included 16 from blood cultures, 1 from CSF and 1 from a placental swab. Eleven of the isolates from humans typed as serotype 4b, 6 typed as serotype 1/2a and 1 typed as serotype 1/2b. Analysis of wgs further subdivided the *Listeria* 4b isolates into Sequence Types (ST)1 (n=3), ST2 (n=3), a single locus variant (SLV) of ST2 (n=1), ST6 (n=3) and ST54 (n=1). The 1/2a isolates were divided into ST18 (n=1), ST37 (n=1), ST121 (n=2), ST155 (n=1) and ST425 (n=1) while the 1/2b isolate was ST87. The wgs data do not suggest a common source link between any of the cases of *L. monocytogenes* diagnosed in Ireland in 2018.

The NSSLRL is working with colleagues in food and veterinary microbiology in Ireland and with colleagues in Europe to build a library of typing data that may help to identify sources of human infection. A critical limiting factor is the availability of human isolates for typing. The total number of human clinical isolates in Ireland per year is very small therefore it is critical that all such isolates are available for typing and we wish to thank colleagues in diagnostic laboratories for the increase in submission of isolates for typing in recent years.

Table 5: Number and serotypes of *Listeria monocytogenes* isolates from human sources received in NSSLRL

Year	Total	4b	1/2a	1/2b	1/2c
2018	18	11	6	1	0
2017	11	2	8	1	0
2016	9	4	4	1	0
2015	19*	10	8	1	0
2014	10	4	6	0	0
2013	7	6	1	0	0
2012	8	5	1	2	0
2011	6	3	3	0	0
2010	4	2	1	0	1
2009	8	4	3	0	1
2008	14	9	4	0	1
2007	12	9	2	0	1
2006	1	0	1	0	0
2005	4	3	1	0	0

Listeria monocytogenes can be subdivided into 13 different serotypes based on their combinations of O and H antigens. However serotypes 4b and the 1/2 group account for the vast majority of human infections.

Shigella species

A total of 92 isolates were referred to the NSSLRL in 2018 for *Shigella* typing. When non-*Shigella*, QC and duplicate isolates were removed a total of 75 *Shigella* isolates were typed. These consisted of 48 *S.sonnei* , 25 *S.flexneri*, and 2 *S.boydii*. The *S.flexneri* isolates were further divided into 7 *S.flexneri* (not serotyped), 2 *S.flexneri* 1b, 1 *S.flexneri* 1c, 8 *S.flexneri* 2a, 3 *S.flexneri* 3a, 3 *S.flexneri* 6 and 1 *S.flexneri* Y variant.

The number of isolates increased each year since 2012 to 2017 from a low point of 20 to 87. There is a reduction in numbers in 2018 (75) compared to 2017 (87) though it is too early to say if this represents a trend towards reduction. It is worth noting that as laboratories changed to use of direct molecular detection of pathogens in faces this appears to have resulted in a striking increase in submission of *Shigella species* for typing. Thus some part of the increase from the low level of 2012

is likely to be related to improved detection however this is unlikely to account for all of the increase. One outbreak in the East of the country contributed significantly to the overall total.

Shigella in the absence of travel history is now strongly associated with young males and as highlighted by outbreaks in Ireland and elsewhere men who have sex with men constitute a specific risk group for sexually transmitted shigellosis.

Antimicrobial susceptibility testing was discontinued on *Shigella* isolates received on or after 1st October 2018 so phenotypic susceptibility results are available only on 54/75 (72%) of isolates. Prediction of resistance is now based on analysis of whole genome sequences. This helps to contain costs and reduce laboratory waste. The majority, 83.3 %, of isolates (n = 45) were multi-drug resistant (three or more different classes of antibiotics). Eight ESBL producing *Shigella* including 5 *Shigella sonnei* (country of infection India (n=1) and no details (n=4), 2 *S.flexneri* 2a (Ireland (n=1) and Spain (n=1)) and 1 *S.flexneri* 3a (Asia) were received in the NSSLRL in 2018.

Analysis of wgs showed five of the ESBL-*Shigella* isolates contained *bla*CTX-M-15, 1 produced *bla*CTX-M-27 and the other *bla*CTX-M-55. Twenty six isolates were resistant to ciprofloxacin. The ciprofloxacin resistant isolates included 20 *Shigella sonnei*, 5 of which were known to be associated with travel to the Indian subcontinent. Many were part of an MSM related outbreak in the East of the country, SH-B/17, (n=8). The proportion of *S. sonnei* isolates resistant to ciprofloxacin is a real concern given that this has increased significantly in recent years. Six *S.flexneri* isolates were resistant to ciprofloxacin including 2 with history of recent foreign travel to the Indian subcontinent and another with contact with someone who had visited India. Two were linked to an MSM related outbreak.

The NSSLRL added azithromycin to its antibiotic panel in October 2013 and in 2018 16/54 isolates exhibited resistance to azithromycin. These included 12 *S.sonnei* and 4 *S.flexneri*. Nine isolates were resistant to multiple antibiotics including azithromycin, ciprofloxacin and trimethoprim.

Twenty seven patients had a recorded history of recent foreign travel, including Europe (n = 5), Africa (n = 7), Australasia (n = 10), Americas (n = 4) and foreign travel with country unknown (n=1). Eleven patients had Ireland recorded as their country of infection while there were no details for 37 patients.

An extended incident/series of linked incidents of transmission of *Shigella* spp. amongst men who have sex with men were noted in the East of the country involving numerous *Shigella* serotypes and antibiograms. This was noticed in 2015 and continued into 2018. In some cases there were no oral options for treatment of the infection and in the case of one group of isolates, ACSuTTmNaCpCtx (n = 2), even the option of a parenteral cephalosporin was not available.

Table 6: Number of *Shigella* isolates received in NSSLRL

Year	Total	*sonnei*	*flexneri*	*boydii*	*dysenteriae*
2018	75	48	25	2	0
2017	87	47	34	2	4
2016	74	42	28	2	3
2015	67	34	27	4	2
2014	45	27	18	0	0
2013	43	23	16	4	0
2012	20	12	6	2	0
2011	30	20	10	0	0
2010	39	17	18	3	0
2009	48	19	24	1	4
2008	43	22	16	5	0
2007	20	5	12	2	1
2006	20	7	12	0	1
2005	13	8	5	0	0

Shigella sonnei has only one serotype while the other *Shigella species* can be can be subdivided into a number of different serotypes and subserotypes based on their lipopolysaccharide antigens.

Figure 2 Summary of *Shigella* isolates typed in NSSLRL from 2005-18

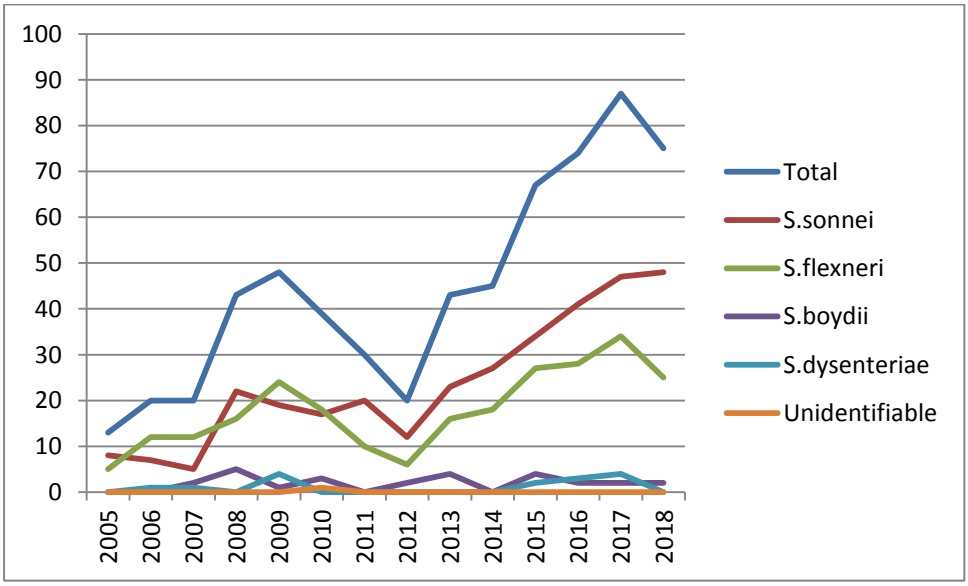


Table 7: Foreign travel history for *Shigella* isolates

Continent	Country	Number
Africa (n = 7)		
	Sudan	2
	Zambia	2
	Morocco	1
	Tanzania	1
	Africa*	1
Europe (n = 5)		
	Spain	4
	UK	1
Australasia (n = 10)		
	India	5
	Nepal	2
	Pakistan	2
	Asia	1
Americas (n = 4)		
	Cuba	2
	Bolivia	1
	Carribean*	1
Foreign travel with country unknown		1

* Country not stated

Appendix: Whole Genome Sequencing

The speed and accuracy of sequencing has increased and the cost has decreased dramatically in recent years. It is now possible to use sequencing of bacterial pathogens in outbreak investigations. The NSSLRL completed the transition to wgs in 2018.

Our primary interest when analysing sequences is to determine relatedness between bacterial isolates in a timely manner.

The most widely used methods are;

1) Whole genome multi locus sequence typing (wgMLST) or a variation such as core genome (cg)MLST.
2) Single nucleotide polymorphisms (SNP) analysis.

wgMLST

MLST was first used in 1998 to type *Neisseria meningitidis* and has since been used to type a huge number of pathogens. As initially developed MLST involved extracting DNA from bacterial isolates, performing separate PCR reactions on a number of internal fragments (450-500 base pairs) of housekeeping genes, purifying the PCR products and sequencing the products using Sanger sequencing. The resultant sequences are then analysed to determine the alleles at each locus. Each time a novel allele is detected it is assigned a new number. The numbering system is sequential so the distance between numbers does not correlate with degree of relatedness. Differences in allele sequences can arise from point mutations, insertions or deletions (Indels), recombination events or a combination of the above. A unique combination of alleles at each locus, an "allelic profile" specifies the sequence type (ST). The MLST allele sequences and allelic profiles are stored in various curated databases worldwide and these are collected by the PubMLST site and made easily accessible [http://pubmlst.org].

Most *Salmonella* serotypes are "monophyletic" this means that they consist of variants of a common ancestral sequence type, e.g. *S*.Enteritidis = ST11 (5,2,3,7,6,6,11), *S*.Typhimurium = ST34 (10,7,12,9,5,9,2), *S*.4,[5],12:i:- = ST19 (10,19,12,9,5,9,2), while other serotypes, e.g. *S*.Newport, have multiple lineages (polyphyletic) and consist of numerous, often unrelated, sequence types, e.g. ST118 (16,42,39, 2,43,45,36) and ST166 (5,14,6,12,5,14,58).

With the advent of WGS an isolates seven gene allele profile and sequence type can be deduced from the genome sequence without having to do the separate PCRs. Also instead of analysing just 7 housekeeping genes the number of genes examined can be greatly increased to look at entire core genomes (genes present in the genomes of the vast majority of that pathogen) cgMLST or whole genomes wgMLST incorporating both core and accessory genomes. This obviously greatly increases the discriminatory power of MLST from the conventional 7 gene MLST schemes.

The wgMLST schemes need constant curation for QC and assigning new allele numbers. A major advantage is that results are easily comparable when laboratories use the same schemes.

Fig. 3 Diagramatic representation of Multi Locus Sequence Typing (MLST) of *S*.Enteritidis.

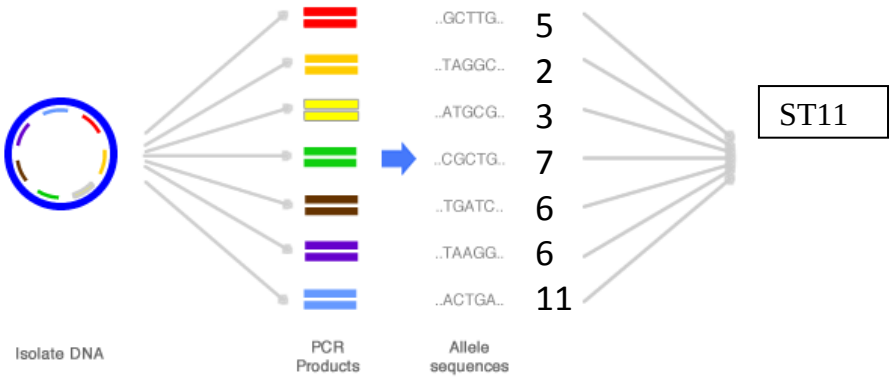


Fig. 4 Variations in allele sequences arising by single point mutations (strain 2, locus A &B), insertions and/or deletions or indels (strain 3 locus A &B) and inversions (strain 4, locus A).

'Locus' (gene)	Strain 1	Strain 2	Strain3	Strain 4
A	ACTAGAGGGAA	ACTAGAGGCAA	ACT-GAGGGTAA	ACGGGAGATAA
	allele 1	allele 2	allele 3	allele 4
B	TAGCCAGGGTC	TAGCAAGGGTC	TAGC---GGTC	TAGGCAGGGTC
	allele 1	allele 2	allele 3	allele 1
C, D, E, etc....	alleles 5,2,8...	alleles 1,4,7...	alleles 1,3,9...	alleles 6,2,9...

SNP analysis

wgMLST only takes account of coding sequences. SNP analysis takes account of mutations throughout the genome. Short reads from isolates would always be compared against closely related reference genomes, e.g. *S*.Enteritidis against a *S*.Enteritidis reference, *S.sonnei* against a *S.sonnei* reference, etc. This method is harder to standardise as results depend on the reference strain used.

Figure 5. Dendrogram of hqSNP analysis of *Shigella sonnei* using BioNumerics software

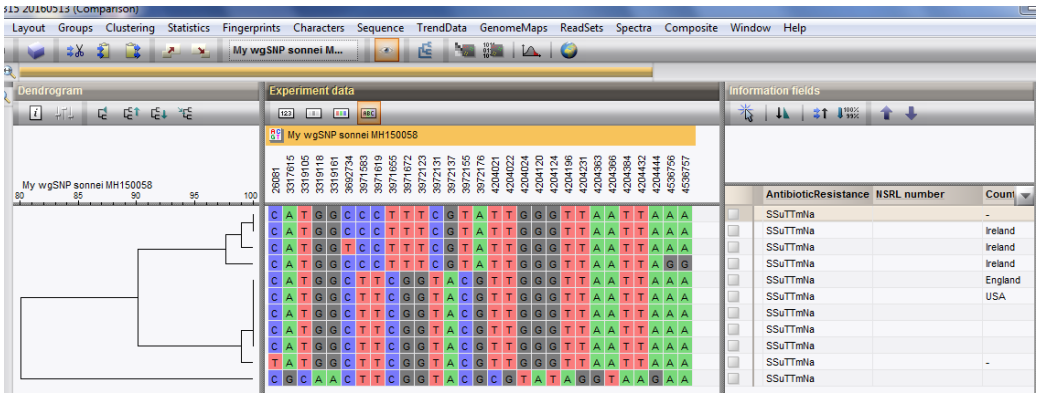
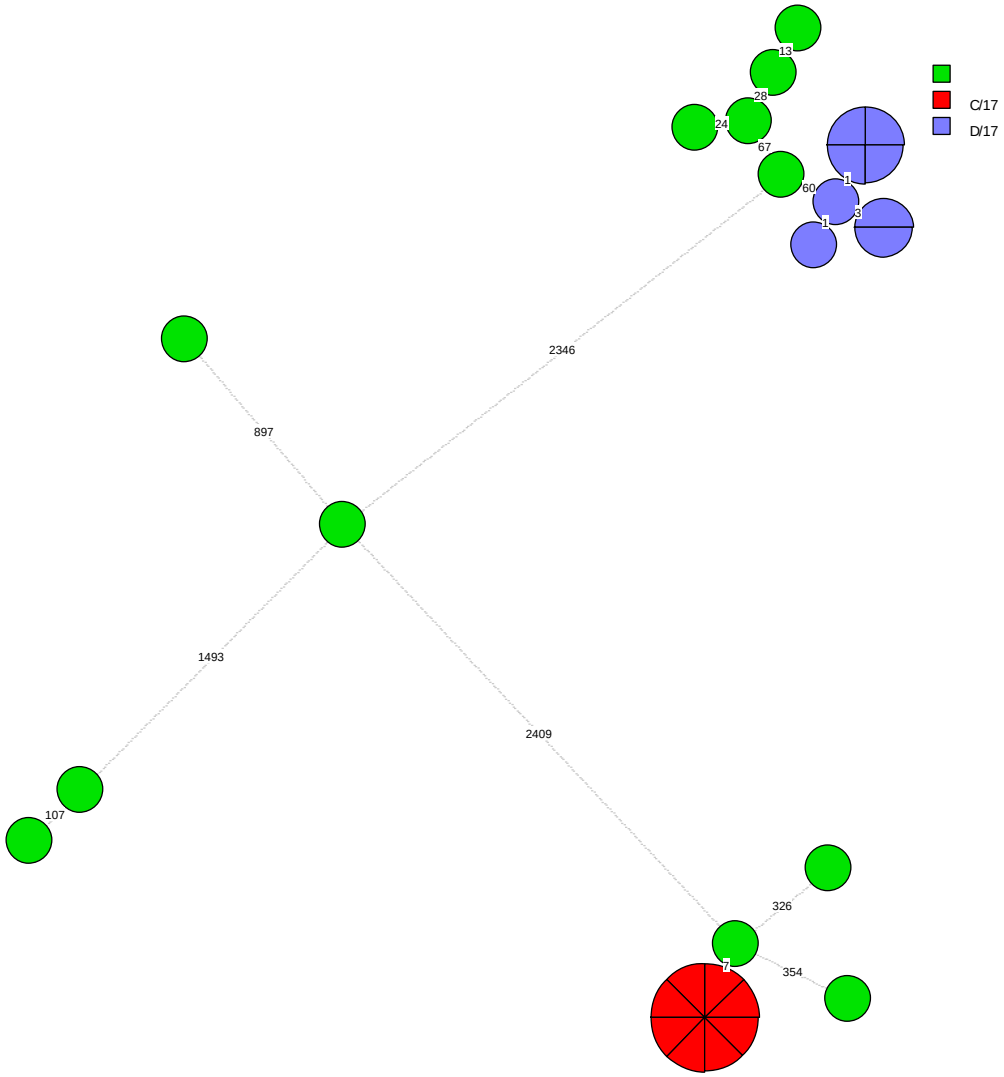


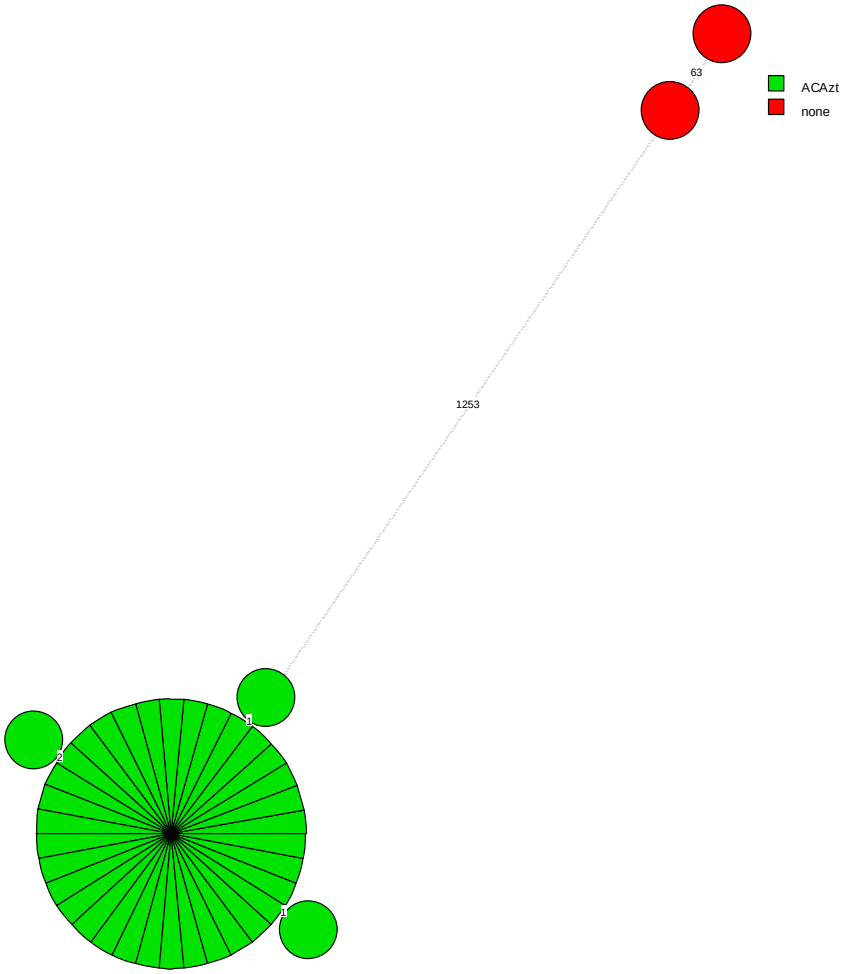
Figure 6. wgs based characterisation of *Salmonella* Newport in Ireland 2015-18.



Legend. Minimum spanning tree (MST) of *Salmonella* Newport core genome MLST from isolates from humans in the NSSLRL from 2015-18 coloured by designated cluster.

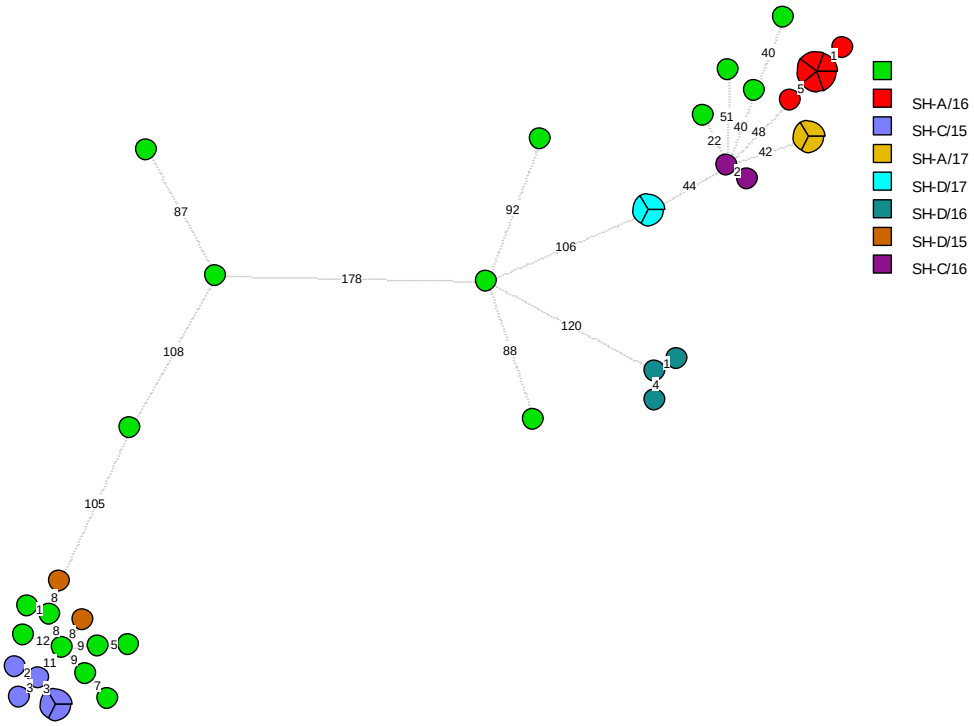
S.Newport (6,8:e,h:2) is a polyphyletic serotype, i.e. isolates with this antigenic structure have originated at different times so all did not evolve from a single ancestor. The numbers on the branches indicates the number of allele differences between isolates. Epidemiologically related isolates should have the same or closely related cg and/or wgMLST profiles. Cluster C/17 isolates were fully susceptible to all antimicrobials tested. Many of the cases were young adults and had a history of travel to a tourist destination island. In contrast the isolates in cluster D/17 had resistance to quinolones and were mainly associated with an older cohort in the East of the country. [The code C/17 is NSSLRL designation for 3rd such cluster identified in the year 2017]

Figure 7. wgs based characterisation of *Salmonella* Brandenburg in Ireland 2015-18.



Legend. cgMLST of *S*.Brandenburg isolates coloured by resistance profile. The outbreak isolates and an isolate from an implicated food were indistinguishable or had just 1 allele difference from each other. There were >1200 allele differences to 2 unrelated isolates.

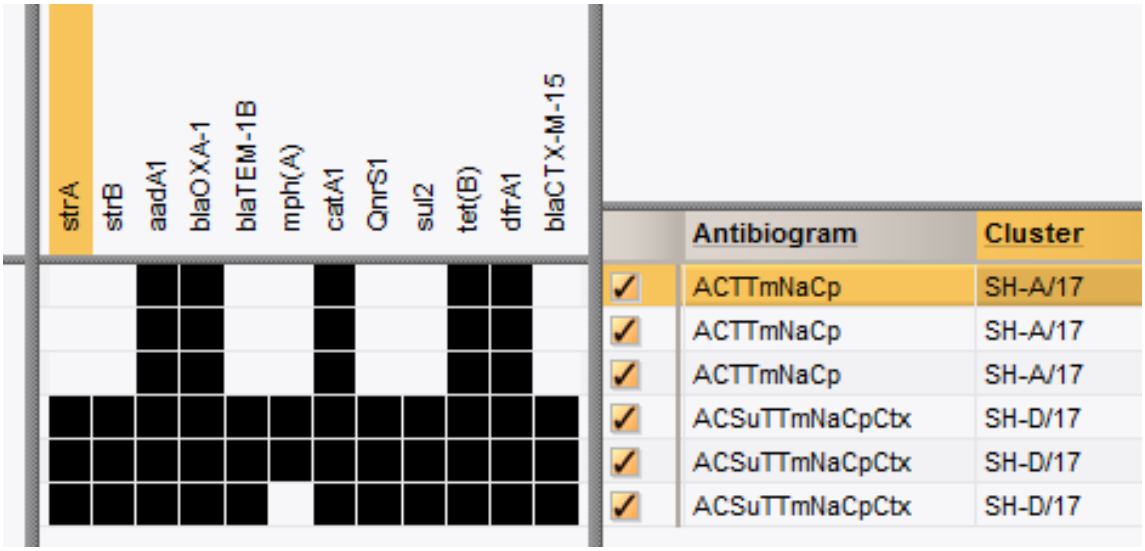
Figure 8. wgs based characterisation of *Shigella flexneri* in Ireland 2015-17.



Legend cgMLST of *Shigella flexneri* 2a isolates from humans in the NSSLRL from 2015-17 grouped by cluster.

Clusters SH-C/15, SH-D/15, SH-A/16, SH-D/16 and SH-D/17 were all clusters associated with msm while cluster SH-A/17 was a family outbreak with a link to India.

Figure 9. Illustration of resistance genes detected in *Shigella flexneri* isolates using Resistance detection software



Legend. Analysis of resistance genes of clusters SH-A/17 and SH-D/17 using resistance detection software incorporated into BioNumerics.