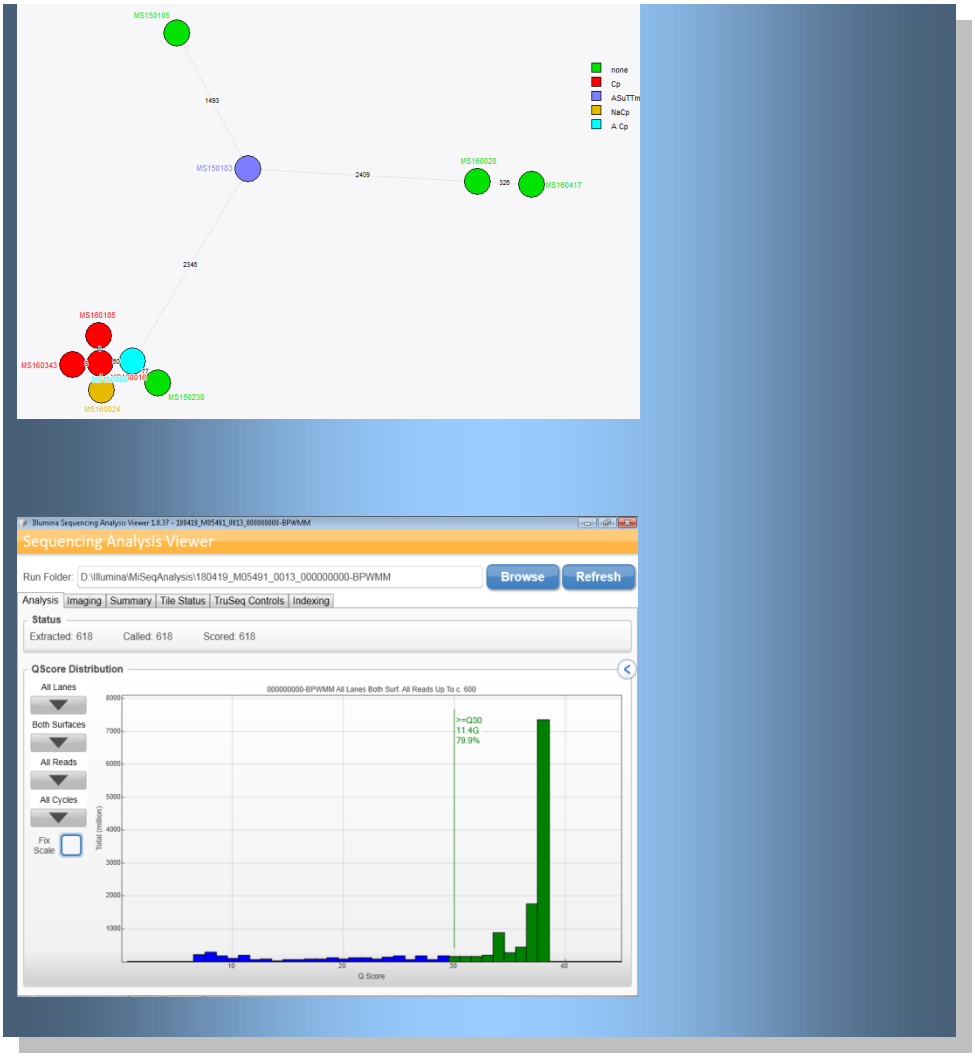
NATIONAL *SALMONELLA, SHIGELLA & LISTERIA* REFERENCE LABORATORY OF IRELAND (HUMAN HEALTH)



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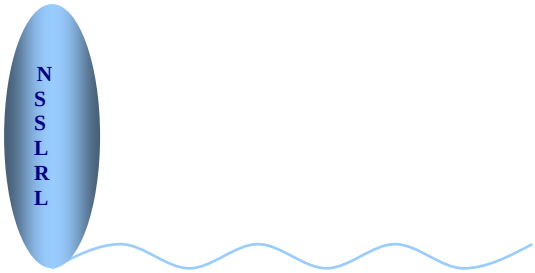

Introduction

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 uses a number of methods (serotyping, antimicrobial susceptibility testing and molecular methods) to characterise isolates. 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.
One of the major challenges that NSSLRL has been addressing is the change from established molecular methods to whole geneome sequencing and bioinformatic analysis. This change has now been implemented

The staff of the reference laboratory would like to acknowledge the support of colleagues in the Information and Communication Technology services at GUH in making the change to wgs. WGS was performed on all isolates of *Salmonella*, *Shigella* and *Listeria monocytogenes* received in 2017. The sequencing was outsourced while we concentrated on developing the analytical expertise however the change to in-house generation of sequence data has been implemented in 2018. In house sequence data has improved turnaround time and is expected to allow the laboratory to dispense with traditional serotyping in 2018.

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.

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].

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 2017, 463 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 393 *Salmonella* isolates were typed. This represents a 6.9% increase in the number of isolates received compared with 2016.

The number of human isolates of *Salmonella* submitted increased by 25.6% compared with 2016 numbers.

There were 388 human clinical isolates, including 347 faecal isolates, 22 from blood (including 10 *S*.Typhi, 3 *S*.Paratyphi A and 1 *S*.Paratyphi B), 6 other invasive isolates, and 12 urine isolates. *S*.Typhimurium (n = 49) and its monophasic variant 4,[5],12:i:- (n = 58) and *S*.Enteritidis (n = 82) 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 (e.g. 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 6 days (range 1-17 days). The number of human *Salmonella* isolates received reached its lowest point in 2014 (n=258) but the annual numbers have increased progressively since then. The number of cases in 2017 is 50% higher than observed in 2014. Data on country of infection are available for 80 to 90% of isolates submitted each year. The percent of isolates associated with foreign travel in 2017 was 84% higher than in 2014 (167 compared with 91). The percent isolates associated with infection in Ireland in 2017 was 29% higher than in 2014 (156 compared with 129). Thus, although there is year to year variation, it appears that the increased number of infections may be mainly related to travel outside of Ireland. In recent years the number of *S.* Typhimurium isolates has decreased and the number of *S.* Enteritidis cases has increased (Table 1).

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

Year	Human	Non-human
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

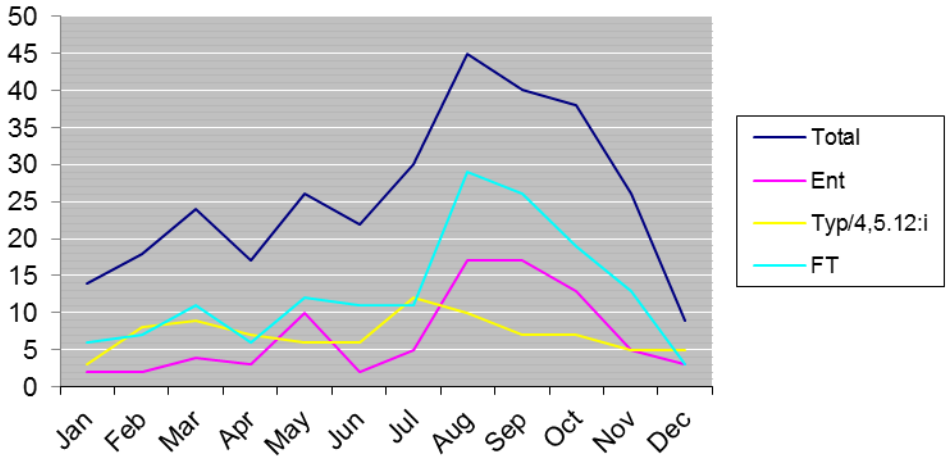
Table 2: Top 15 serotypes of Human Isolates (inc typhoidal)

Serotype	Frequency	%
Enteritidis	82	21.1
Monophasic Typhimurium[1]	58	14.9
Typhimurium	49	12.6
Brandenburg	36	9.3
Newport	17	4.4
Typhi	14	3.6
Infantis	9	2.3
Stanley	9	2.3
Agona	8	2.1
Virchow	6	1.5
Weltevreden	6	1.5

Paratyphi A	5	1.3
Bredeney	4	1.0
Chester	4	1.0
Agama	3	0.8
Durham	3	0.8
Hadar	3	0.8
Java	3	0.8
Kentucky	3	0.8
Others	66	17.0
Total	388	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 27.5 % of all cases of *Salmonella*.

S.Enteritidis

S.Enteritidis accounted for 21.1% of all cases of *Salmonella*.

***Salmonella* Typhi and Paratyphi**

Fourteen isolates of *S*.Typhi, 5 of *S*.Paratyphi A and 1 *S*.Paratyphi B isolate were received. A history of recent travel was recorded for 11 of the *S*.Typhi isolates; all to the Indian subcontinent, while a child who acquired the infection in Ireland had parents from the Indian subcontinent. Four of the *S*.Paratyphi A had travel to the Indian sub-continent. The *S*.Paratyphi B isolate had a reported history of recent travel to South America.

Antimicrobial resistance

Almost half (192 of 388 isolates, 49.5%) were susceptible to all antimicrobial agents tested. Thirty one point four percent (31.4%) of isolates (n = 122) were multi-drug resistant (three or more different classes of antibiotics). Thirty point three percent of isolates that were multi-drug resistant, 30.3% (n = 37) had the profile of resistance to ampicillin, sulphonamide and tetracycline (ASuT) and were mainly monophasic S. Typhimurium. The NSSLRL changed antibiotic susceptibility testing panel on 1st Oct 2016. Since that time the new plate does not include streptomycin as this drug is not clinically relevant and has proved unsatisfactory as an epidemiological marker.

Three extended spectrum beta-lactamase (ESBL) producing isolates were detected. All were associated with recent foreign travel; a monophasic Typhimurium had travel history to Thailand, a *S*. Kentucky with travel to Malta and a *S*. Enteritidis with travel to New Zealand.

Seventy four isolates of *Salmonella* resistant to ciprofloxacin were detected (19.1%). It is worth noting that the EUCAST interpretive criteria for resistance to ciprofloxacin for *Salmonella* changed on January 1st 2014 and isolates with ciprofloxacin MIC of > 0.06 mg/l are now reported as resistant. By current EUCAST criteria therefore 15.9% (n = 55) of *Salmonella* isolates from 2013 would be considered ciprofloxacin-resistant. Therefore 19.1% represents a small increase in ciprofloxacin resistance compared with 2013.

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.

Three such isolates were typed in the NSSLRL in 2017, 1 of which had a history of foreign travel to Malta, another to Algeria and another to India.

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 38 patients including 35 from a *S*.Brandenburg outbreak, and 1 with foreign travel to the Phillipines and another to Tunisia. No isolates exhibited tigecycline resistance.

Travel related infection

A history of recent foreign travel was recorded in 167 of the 388 (43%) human cases of infection (Table 3). Ireland was noted as the country of infection in 40.2 % (n = 156) of cases while 16.8 % (n = 65) had no country of infection recorded. Spain, Thailand and Portugal were the most commonly recorded travel destinations. *S*. Enteritidis accounted for a high proportion of isolates associated with travel to Spain (40.5%) and Portugal (60%) while *S*.Stanley was strongly associated with travel to Asia (55.5%), i.e. 5/9 isolates. Fifty one of 82 (62.2 %) *S*.Enteritidis isolates were associated with foreign travel compared to 32.7 % 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 = 76)		
	Spain	37
	Portugal	10
	Italy	6
	Poland	5
	United Kingdom	4
	Hungary	3
	Germany	2
	Greece	2
	Andorra	1
	Bulgaria	1
	Croatia	1
	France	1
	Kosovo	1
	Malta	1

	Turkey	1

Africa (n = 18)

	Morocco	5
	Nigeria	3
	Kenya	2
	Algeria	1
	Botswana	1
	Malawi	1
	Mauritius	1
	Tanzania	1
	Tunisia	1
	Uganda	1
	Zambia	1

Australasia (n = 61)

	Thailand	15
	India	9
	Vietnam	6
	Bangladesh	5
	China	4
	U.A.E.	4
	Pakistan	3
	Australia	2
	Indonesia	2
	The Philippines	2
	Vietnam & Cambodia	2
	Middle East	1
	Nepal	1
	New Zealand	1
	U.A.E & Australia	1
	Vietnam & Thailand	1
	Asia*	2

Americas (n = 12)

	Cuba	2
	Dominican Republic	2

Haiti	2
USA	2
Caribbean	1
Columbia	1
Mexico	1
South America*	1
Foreign travel with country unknown	1

* Country not stated

Clusters

Forty three clusters of cases involving 145 isolates were identified in 2017. *S*. Typhimurium/monophasic S. Typhimurium was involved in 17 clusters (40 isolates) while *S*. Enteritidis was implicated in 11 clusters (30 isolates).

Three of the clusters (6 patients) were family outbreaks, i.e. all patients affected were from one family.

A large outbreak of *S*.Brandenburg involving 35 confirmed cases was associated with a restaurant/bar. An isolate of Salmonella from a food consumed at the premises matched the outbreak strain by whole genome sequencing.

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

Animal Contact

A history of animal contact was recorded for 69 patients with salmonellosis including contact with reptiles, birds, fish, horses, dogs and farm animals. Dogs were the most common contact animal (n = 41) while contact with cats was less common (n = 14).

Strong links included patients with *S*.Luke and *S*.Newport associated with reptiles and farmers with *S*.Agama and *S*.4,[5],12:i:-.

In total 14 isolates of *Salmonella* (approx. 3.6 % of all human cases) were associated with contact with exotic animals although in many cases 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, e.g. recent history of foreign travel, consumption of particular food products, etc. 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 perform full characterisation on non-human isolates from official food laboratories. In 2017, 5 isolates of *Salmonella* of non-human origin were submitted to the NSSLRL. The majority of isolates were from herbs and spices (n = 4) while 1 was from a food sample associated with an outbreak.

Table 4 Serotypes among non-human isolates

Serotype	Frequency	%*
Anatum	1	20
Brandenburg	1	20
Enteritidis	1	20
Typhimurium	1	20
Unnamed	1	20
Total	5	100%

Antimicrobial Resistance among non-Human isolates

Antimicrobial susceptibility testing was performed on the 5 non-human isolates. Three were susceptible to all antimicrobial agents while 2 were multi-drug resistant (three or more different classes of antibiotics).

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

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

Year	Total	4b	1/2a	1/2b	1/2c
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.

The NSSLRL received 11 *Listeria monocytogenes* isolates from human clinical samples in 2017. These included 8 from blood cultures and 3 from CSFs. Two of the isolates from humans typed as serotype 4b, 8 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 and ST6 and divided the 1/2a 8 isolates ST7, ST8, ST37 (two isolate,mother-infant pair), ST204, ST226, ST425 and ST504. The diversity apparent from the wgs data allows us to conclude that there is unlikely to be any common source link between any of the cases of *L. monocytogenes* diagnosed in Ireland in 2017. This does not however exclude links at the European level and it is with this in mind that pan European cooperation on whole genome sequencing of *L. monocytogenes* is important as reflected in the publication at the following link. https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2018.23.33.1700798

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 appeal for all isolates to be forwarded for typing.

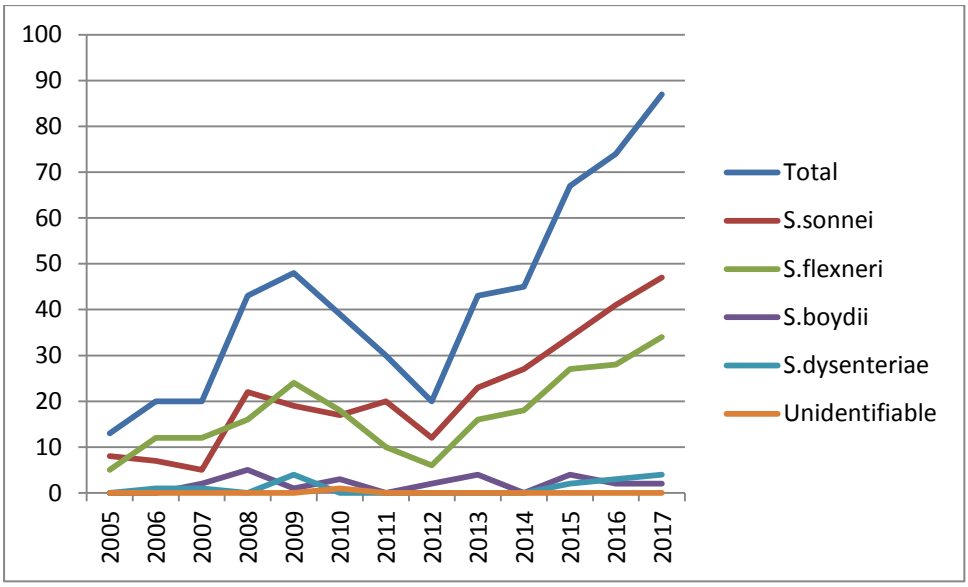
Shigella species

Table 6: Number of *Shigella* isolates received in NSSLRL 2005-17

Year	Total	*sonnei*	*flexneri*	*boydii*	*dysenteriae*
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 2005-17



A total of 115 isolates were referred to the NSSLRL in 2017 for *Shigella* typing. When non-*Shigella*, QC and duplicate isolates were removed a total of 87 *Shigella* isolates were typed. These consisted of 47 *S.sonnei* , 34 *S.flexneri*, 4 *S.dysenteriae* and 2 *S.boydii*. The *S.flexneri* isolates were further divided into 5 *S.flexneri* 1b, 3 *S.flexneri* 1c, 1 *S.flexneri* 2, 17 *S.flexneri* 2a, 3 *S.flexneri* 2b, 2 *S.flexneri* 3b, 1 *S.flexneri* 4, 1 *S.flexneri* 5b and 1 *S.flexneri* 6.

This is the highest number of *Shigella species* ever submitted to the NSSLRL. The number of isolates has increased each year since 2012 from a low point of 20 to 87 in 2017. This represents an increase of more than 400% in five years although the increase is from a very low base. 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. This is most likely explained by the increased sensitivity of the method compared to conventional culture. Thus some part of the increase in 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 an outbreak in the east of the country men who have sex with men constitute a specific risk group for sexually transmitted shigellosis.

The majority, 82.8 %, of isolates (n = 72) were multi-drug resistant (three or more different classes of antibiotics). Nine ESBL producing *Shigella* including 5 *Shigella sonnei* (country of infection Pakistan (n=2), India (n=1) and Egypt (n=2)), 3 *S.flexneri* 2a (Ireland (n=2) and no details (n=1))

and 1 *S.boydii* (no details) were received in the NSSLRL in 2017. Analysis of wgs showed all of the ESBL-Shigella isolates contained *bla*CTX-M-15. Thirty two isolates were resistant to ciprofloxacin. The ciprofloxacin resistant isolates included 22 *Shigella sonnei*, 2 of which were known to be associated with travel to the Indian subcontinent. The majority were part of an MSM related outbreak in the East of the country, SH-B/17, (n=14), and a point-source outbreak in the South of the country. The proportion of *S. sonnei* isolates resistant to ciprofloxacin is a real concern given that this has increased significantly in recent years. With international colleagues we have contributed to a publication that indicates that a clonal group associated with ciprofloxacin-resistance has emerged and spread globally in recent years (see publications listed below). Ten *S.flexneri* isolates were resistant to ciprofloxacin including 1 with history of recent foreign travel to the Indian subcontinent and a family outbreak (n=3) in a family of Indian extraction. Others (n=5) were linked to an MSM related outbreak.
The NSSLRL added azithromycin to its antibiotic panel in October 2013 and in 2017 21/87 isolates exhibited resistance to azithromycin. These included 16 *S.sonnei*, 3 *S.flexneri* 2a and 2 *S.flexneri* 1c.

Twenty seven patients had a recorded history of recent foreign travel, including Europe (n = 1), Africa (n = 11) and Australasia (n = 6). Thirty six patients had Ireland recorded as their country of infection while there were no details for 24 patients.

Table 7: Foreign travel history for *Shigella* isolates

Continent	Country	Number
Africa (n = 11)		
	Egypt	2
	Nigeria	2
	Cameroon	1
	Kenya	1
	Malawi	1
	Mali	1
	Morocco	1
	Sudan	1
	Tanzania	1
Europe (n = 1)		
	Spain	1
Australasia (n = 6)		

India	3
Pakistan	3

Americas (n = 9)

Brazil	1
Brazil & Mexico	1
Central America*	1
Costa Rica	1
Cuba	1
Ecuador	1
Peru	1
South America*	1

* Country not stated

Of particular concern in relation to antimicrobial resistance are groups of isolates with polymicrobial resistance associated with MSM. In the most extreme example there were 3 cases of infection with *S. flexneri* resistant ampicillin, azithromycin, ceftriaxone, ciprofloxacin, sulfamethoxazole, tetracycline and trimethoprim. With this profile of resistance there are essentially no practical options for oral treatment of infection in those patients who require treatment and even parenteral third generation cephalosporins are not a suitable option.

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. Analysis of whole genome sequences (WGS) is rapidly replacing conventional phenotypic. The NSSLRL made substantial progress in conversion to wgs based typing in 2017 and expects to complete the transition 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 accesible [http://pubmlst.org].

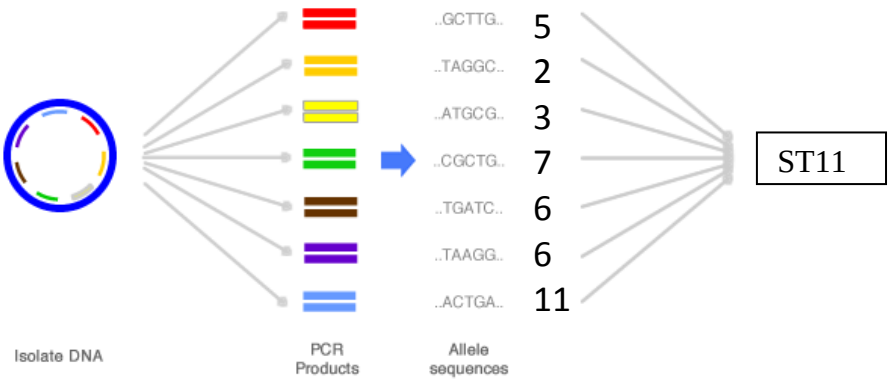


Fig. 3 Diagramatic representation of Multi Locus Sequence Typing (MLST) of *S*.Enteritidis.

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).

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 = ST19 (10,7,12,9,5,9,2), *S*.4,[5],12:i:- = ST34 (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.

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.

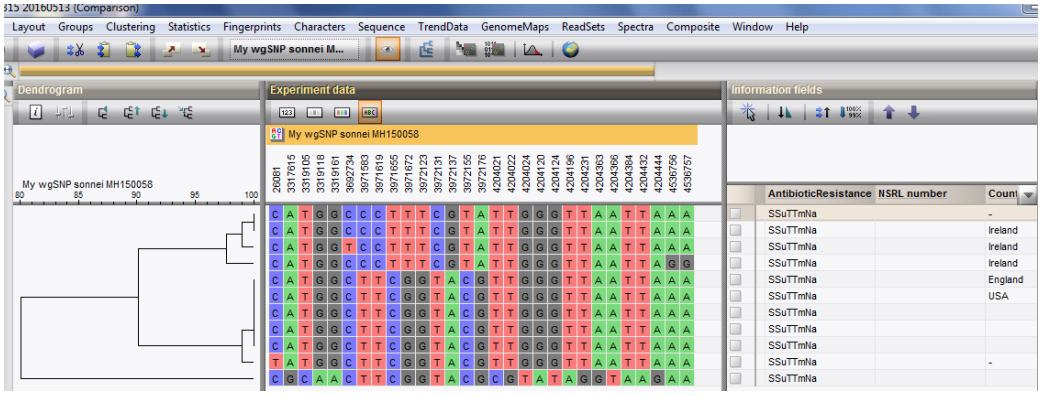


Fig. 5 Dendrogram of hqSNP analysis of *Shigella sonnei* using BioNumerics software.

WGS in the NSSLRL

In 2016 the NSSLRL did not have a sequencer so DNA was extracted and quantified and sent to outside contractors for WGS. WGS (PHE, Colindale), followed by bioinformatics analysis, was performed on selected *Salmonella* and *Shigella* isolates from 2015 and all non-duplicate isolates of *Salmonella* and *Shigella* received from 1st January 2016. A sequencing machine was acquired in 2017 and in house sequencing is currently being validated for 2018.

DNA was extracted from all *Listeria* isolates received from human sources in the NSSLRL from 2010 onwards and sent to the ECDC sequencing contractor as part of the ECDC European *Listeria* Typing Exercise (ELiTE) project.

Bioinformatics analysis was performed primarily with BioNumerics software (Applied Maths).

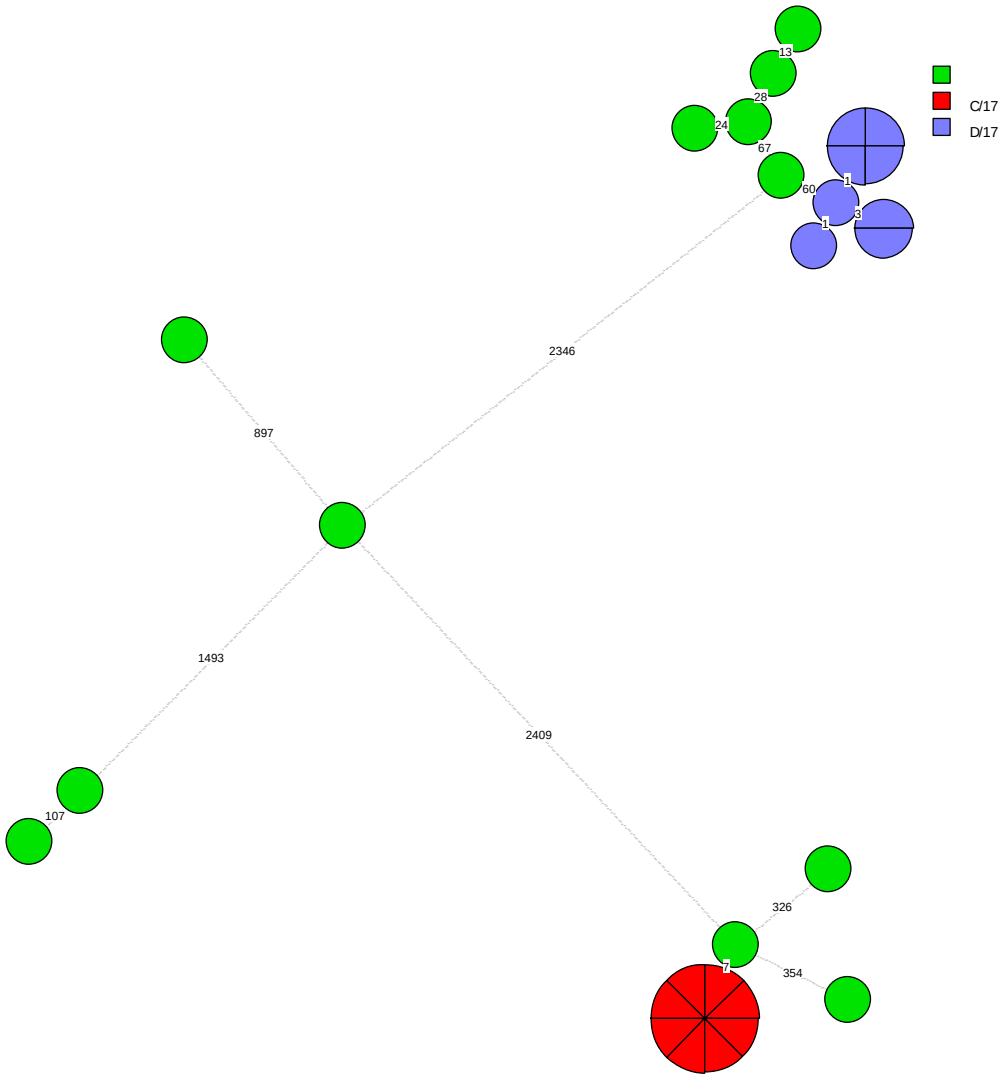


Fig. 6 Minimum spanning tree (MST) of *Salmonella* Newport core genome MLST from isolates from humans in the NSSLRL from 2015-18 coloured by designated cluster.

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]

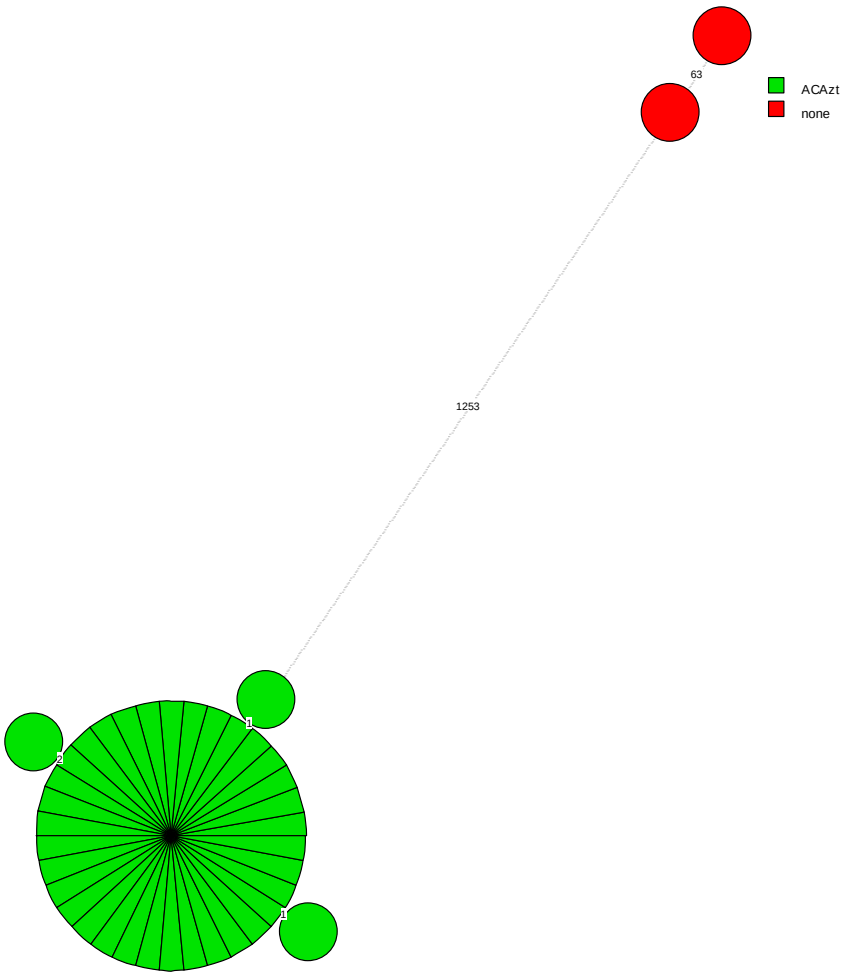


Fig. 7 cgMLST of *S*.Brandenburg isolates coloured by resistance profile. The outbreak isolates and an isolate from an implicated food were indistinguishable or had just 1 allele difference from each other. There were >1200 allele differences to 2 unrelated isolates.

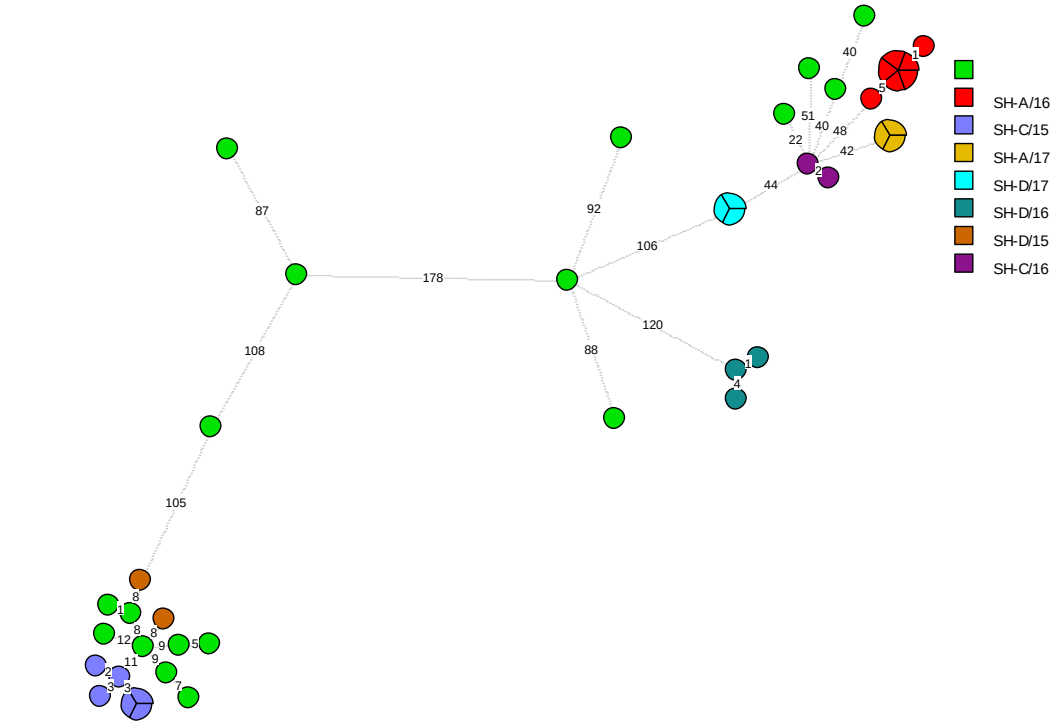


Fig. 8 cgMLST of *Shigella flexneri* 2a isolates from humans in the NSSLRL from 2015-17 grouped by cluster.

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.

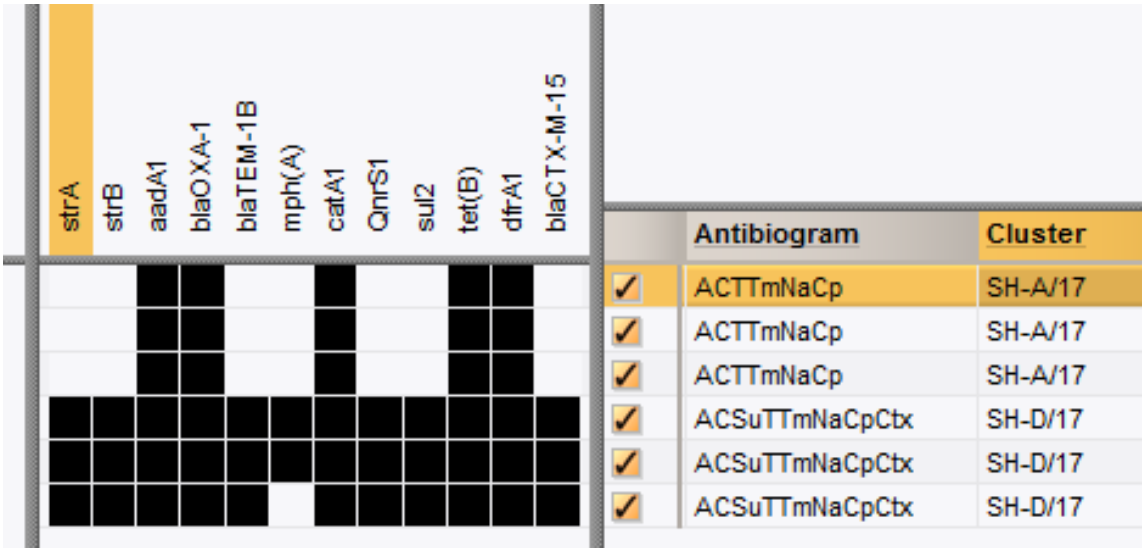


Fig. 9 Analysis of resistance genes of clusters SH-A/17 and SH-D/17 using ResFinder software incorporated into BioNumerics.

NSSLRL Publications and Presentations 2017

Talks/Poster Presentations

Investigation of a Multi-Species outbreak of Shigellosis Associated with Men who have Sex with Men. LabCon. Galway, Mar 31st. 2017.

Antimicrobial Susceptibility testing of Campylobacter species from Humans in Ireland. LabCon. Galway, Mar 31st. 2017.

Whole Genome Sequencing and Antimicrobial Resistance. Genomics in Foodborne Pathogen Surveillance and Outbreak Investigation. University of the Basque Country, Vitoria-Gasteiz , Spain. Jul 12th 2017.

Analysis of WGS in the NSSLRL. St. James Hospital, Dublin. 19th July 2017.

Comparison of Analysis of WGS and Conventional Techniques for detection of Salmonella Clusters. MedVetNet. Guilford, UK. July 27th 2017.

A Bluffers Guide to WGS. Irish Molecular Diagnostics Network. Hilton Hotel, Dublin. Oct 6th 2017.

Cluster Detection using WGS in the NSSLRL. WGS in Food Safety and Public Health. Crowne Plaza, Dublin. Nov 28th 2017.