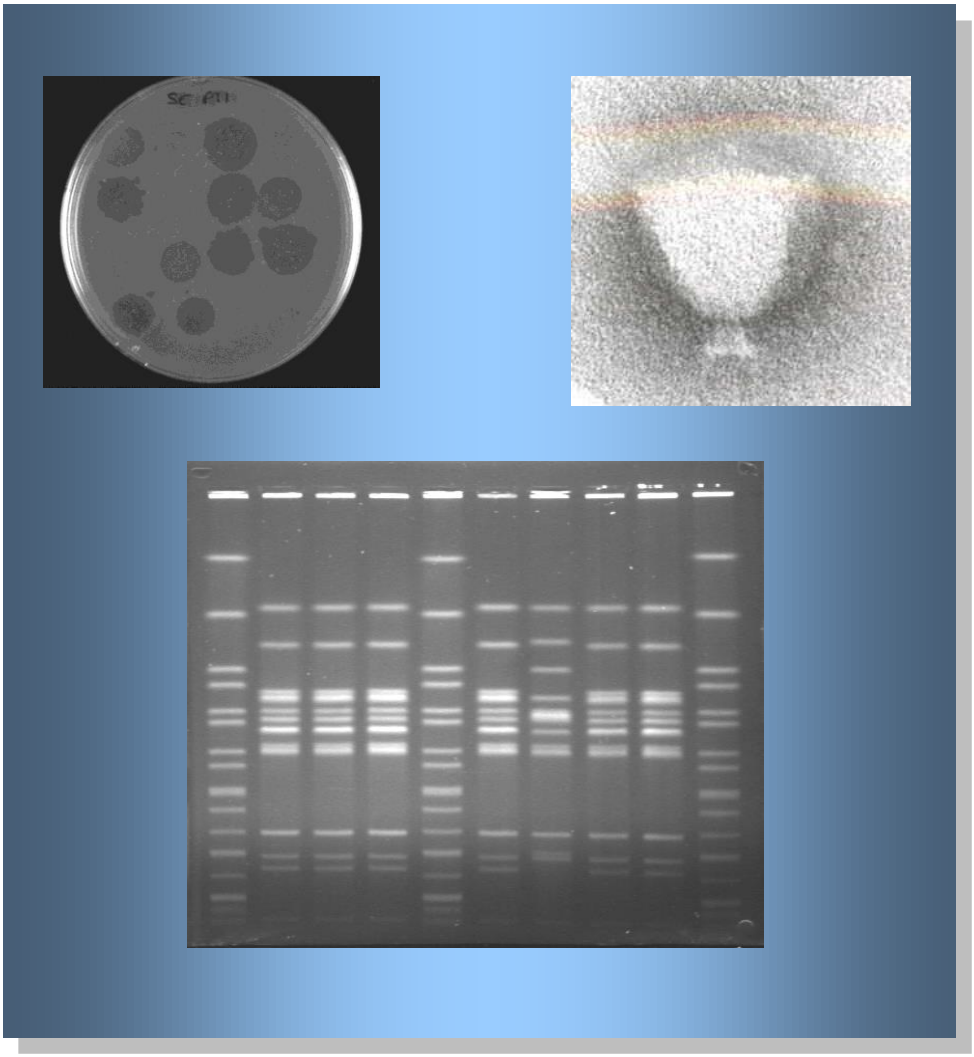
NATIONAL *SALMONELLA, SHIGELLA & LISTERIA* REFERENCE LABORATORY OF IRELAND (HUMAN HEALTH)



ANNUAL REPORT FOR 2016

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 2 WTE scientific staff representing a reduction from 3 staff working in the service until 2011. 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. One effect of this process is that this is the last annual report that will include phage typing results as the method has now been retired as it has become redundant given advances in sequencing technology. Likewise Pulsed Field Gel Electrophoresis (PFGE) and Multi-locus VNTR Analysis (MLVA) are now redundant.

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 2016. The sequencing was outsourced while we concentrated on developing the analytical expertise however the change to in-house generation of sequence data will begin in 2017. In house sequence data will improve turnaround time.

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 2016, 429 isolates 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 377 *Salmonella* isolates were typed. This represents a 30.6% decrease in the number of isolates received compared with 2015. This reduction is related to a reduction in the number of non-human isolates submitted for specifically for phage typing. The number of human isolates submitted increased by 8% compared with 2015 numbers.

There were 309 human clinical isolates, including 274 faecal isolates, 20 from blood (including 7 *S*.Typhi, 5 *S*.Paratyphi A and 1 *S*.Paratyphi B), 8 other invasive isolates, and 7 urine isolates. *S*.Typhimurium (n = 44) and its monophasic variant 4,[5],12:i:- (n = 41) and *S*.Enteritidis (n = 83) 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 2-16 days). The number of human *Salmonella* isolates received is less than half that observed when the laboratory was established in 2000 (Table 1). The lowest number was observed in 2014 (n=258) but there appears to be a trend towards a modest increase in the last 2 years. .

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

Year	Human	Non-human
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

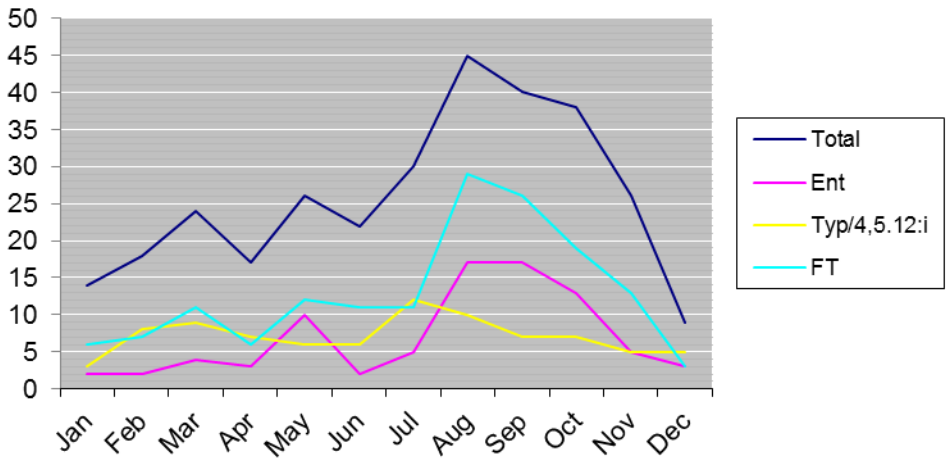
2006	430	308
2005	357	494
2004	420	650
2003	486	634
2002	394	540
2001	508	574
2000	636	21

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

Serotype	Frequency		%
Typhimurium	44		14.2
Monophasic Typhimurium[1]	41	13.3	
Enteritidis	83		26.9
Infantis	11	3.6	
Typhi	10	3.2	
Hadar	7	2.3	
Stanley	7	2.3	
Agona	6	1.9	
Kentucky	6	1.9	
Newport		6	1.9
Paratyphi A	6	1.9	
Saintpaul	6	1.9	
Braenderup	5	1.6	
Java	5	1.6	
Anatum	4	1.3	
Others	62	20.1	
Total	309	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*. Phage type DT193 was the most common (26%) phage type among human isolates in 2016. Phage types Untypable* accounted for 11 %, DT104 5.9 %, DT120 9.4 % and DT104b accounted for 1.2 % of the total number of *S*.Typhimurium/4,[5],12:i:- isolates. The proportion of isolates accounted for by phage type DT104b at 1.2% is very low compared compared with the peak of 20% in 2008. The proportion of isolates accounted for by phage type DT104 is also low at 5.9% compared with the peak of 20.1% in 2008.

* Does not react with typing phages.

S.Enteritidis

S.Enteritidis accounted for 26.9% of all cases of *Salmonella*. The predominant phage types were PT8 (34.9%), PT3 (12%), PT1 (10.8%), PT21 (7.2%) and PT2 (7.2%).

Salmonellosis Typhi and Paratyphi

Ten isolates of *S*.Typhi, 6 of *S*.Paratyphi A and one *S*.Paratyphi B isolate were received. A history of recent travel was recorded for all of the *S*.Typhi isolates; 8 to the Indian subcontinent, 1 with Asia (including India) and 1 to the Philippines. All the *S*.Paratyphi A had travel to Asia, 5 to the Indian sub-continent and 1 to Indonesia. The *S*.Paratyphi B isolate had no reported history of recent travel.

Antimicrobial resistance

More than half (167 of 309 isolates, 54%) were susceptible to all antimicrobial agents tested. Twenty five point nine percent (25.9%) of isolates (n = 80) were multi-drug resistant (three or more different classes of antibiotics). Twenty five point nine percent (25.9%) of isolates that were multi-drug resistant, 8.4% (n = 26) had the profile of resistance to ampicillin, streptomycin, sulphonamide and tetracycline (ASSuT or ASuT) and were mainly monophasic S. Typhimurium. The NSSLRL changed antibiotic testing panel on 1st Oct 2016 and 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. These included an *S*.Agona isolate with mixed ESBL producing and non-ESBL producing populations. This most likely reflects populations with and without a specific plasmid. The second ESBL isolates was a monophasic *S*. Typhimurium (4,[5],12:i:-) and the third a *S*.Unnamed which did not express any O antigens. None of these isolates were associated with a record of recent foreign travel. In contrast, of the two AmpC producing *Salmonella,* a *S*. Anatum isolates were received from a patient with travel to Spain while an AmpC producing *S*.Infantis isolate was received from a patient with travel to the Phillipines.

Seventy-six isolates of *Salmonella* resistant to ciprofloxacin were detected (24.6%). 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.

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. Six such isolates were typed in the NSSLRL in 2016, 1 of which had a history of foreign travel to Spain, another to Pakistan and another to the Philippines.

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 6 patients, including 1 with foreign travel to Thailand and another to Malaysia. No isolates exhibited tigecycline resistance.

Travel related infection

A history of recent foreign travel was recorded in 154 of the 309 (49.8%) human cases of infection (Table 3). Ireland was noted as the country of infection in 42.4 % (n = 131) of cases while 8 % (n = 24) had no country of infection recorded. Spain, Thailand, India and Poland were the most commonly recorded travel destinations. *S*. Enteritidis accounted for a high proportion of isolates associated with travel to Spain (36.8%) and Poland (n = 81.8) while *S*.Stanley was strongly associated with travel to Asia (85.7%), i.e. 6/7 isolates. Fifty four of 83 (65 %) *S*.Enteritidis isolates were associated with foreign travel compared to 25.9 % 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 = 78)		
	Spain	38
	Poland	11
	Turkey	5
	Hungary	4
	Portugal	4
	Czech Republic	3
	Bosnia	2
	Greece	2
	Belarus	1
	Italy	1
	France	1
	Germany	1
	The Netherlands	1
	Malta	1
	United Kingdom	2
	Europe*	1
Africa (n = 11)		
	Morocco	2
	Sudan	2
	Ethiopia	1
	Ghana	1

	Kenya	1
	Nigeria	1
	South Africa	1
	S.Africa/Mozambique	1
	Zambia	1
Australasia (n = 58)		
	Thailand	13
	India	12
	Pakistan	9
	The Philippines	5
	Indonesia	2
	Sri Lanka	2
	Vietnam & Cambodia	2
	Malaysia	1
	Cambodia	1
	China	1
	Iran	1
	Israel	1
	Lebanon	1
	U.A.E.	1
	Saudi Arabia	1
	Thailand & Cambodia	1
	Thailand& Indonesia	1
	Vietnam	1
	Asia*	2
Americas (n = 5)		
	Brazil	2
	Venezuela	1
	Mexico	1
	South America*	1
Foreign travel with country unknown		2

* Country not stated

Clusters

Eighteen clusters of cases involving 40 isolates were identified in 2016. *S*. Typhimurium/monophasic S. Typhimurium was involved in 7 clusters (14 isolates) while *S*. Enteritidis was implicated in 7 clusters (17 isolates).

Eleven of the clusters (25 patients) were family outbreaks, i.e. all patients affected were from one family.

The outbreak of *S*. Typhimurium phage type DT8 that began in August 2009 continued through 2010 and 2011. This phage type is associated with ducks and several of the cases were linked epidemiologically with ducks or consumption of duck eggs. However the number of DT8 isolates from human cases typed in the NSSLRL decreased from 28 in 2010 to 9 in 2011, just 2 in 2012, 3 in 2013, 2 in 2014, 2 in 2015 and 4 in 2016. This suggests that the control procedures put in place are working, leading to reduced burden of human infection from this source.

The NSSLRL liaises with the European Centre for Disease Control (ECDC) in the investigation of outbreaks that may have an international dimension. PFGE images and analysis from *Salmonella* and *Listeria* isolates are also uploaded to a centralised ECDC database where they can be compared with isolates from other countries to check for matches.
One such outbreak in 2016 was an international outbreak of *S*.Enteritidis considered to be associated with eggs from Poland. There we no cases related to this outbreak in Ireland.
https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/rapid-outbreak-assessment-Salmonella-Enteritidis-7-mar-2017.pdf

Animal Contact

A history of animal contact was recorded for 71 patients with salmonellosis including contact with reptiles, birds, fish, horses, dogs and farm animals. Dogs were the most common contact animal (n = 42) while contact with cats was less common (n = 16).

Strong links included patients with *S*. Denver, *S*.Infantis, *S*.Hadar, *S*.Apapa and *S*.Enteritidis PT8 associated with reptiles and a farmer with *S*. Typhimurium DT104 in contact with a calf that had *Salmonella*.

. In total 12 isolates of *Salmonella* (approx. 4 % 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/

Among people that had a recorded history of living on farms or working with farm animals (n = 12), *S*. Typhimurium (n = 1) and its monophasic variant 4,[5],12:i:- (n= 4) predominated.

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. Up to end of 2016 it also performed phage typing on isolates identified as serotypes *S*. Enteritidis or *S*. Typhimurium by the Central Veterinary Research Laboratory. In 2016, 68 isolates of *Salmonella* of non-human origin were submitted to the NSSLRL. This represents a decrease of 73.5 % in the number of non-human isolates received in 2016. The majority of isolates were from swine (n = 26), poultry (n = 19) and bovine (n = 10) sources. *S*. Typhimurium/monophasic *S*. Typhimurium (n = 44) and *S*.Enteritidis (n = 14) were the most prevalent serovars (Table 6).

Table 4 Serotypes among non-human isolates

Serotype		Frequency	%*
Serotyped by CVRL[1]	54	79.4	
Enteritidis	4	5.9	
Infantis	3	4.4	
Others	7	10.3	
Total	68		100%

* Approximate figures

[1] These are Typhimurium/monophasic *S.*Typhimurium (n = 44) and Enteritidis (n = 10) isolates serotyped at the CVRL and submitted to the NSSLRL for phage typing only.

***Salmonella* serotypes and correlation with Human Infection**

S.Typhimurium

S.Typhimurium and its monophasic variant 4,[5],12;i:- accounted for 27.5% of all non-human isolates and were isolated from a variety of sources predominantly swine (n = 26) but also including bovine (n = 6) and poultry (n = 7) sources. Phage types DT193 (n = 7), DT104 (n = 6), DT104b (n

= 4), DT120/DT120 low (n = 4) and Untypable (n = 2), were the most common phage types from swine. Phage type DT193 (n = 3) was the most common phage type among bovine isolates. Phage type RDNC (n = 3) and DT8 (n = 2) were the most common phage type among poultry isolates.

Salmonella Enteritidis.

Fourteen *S*. Enteritidis isolates were received from non-human sources in the NSSRL in 2016, 10 from poultry, 2 from bovine and 2 from zoo animals. It is of interest that *S*. Enteritidis is rarely detected from poultry or poultry products in Ireland but that *S.* Enteritidis is the first or second most common serovar isolated from human infections each year. In this context it is worth noting that a history of travel outside of Ireland is reported in relation to 65% of *S.* Enteritidis cases compared with only 25.9 % of *S.* Typhimurium/monophasic *S*. Typhimurium cases in 2016.

Sources of Isolates

Swine

The 26 isolates received from swine were *S*.Typhimurium and its monophasic variant 4,5,12:i:- . Phage types DT193 (n = 7), DT104 (n = 6), DT104b (n = 4), DT120/DT120 low (n = 4) and Untypable (n = 2), were the most common phage types from swine.

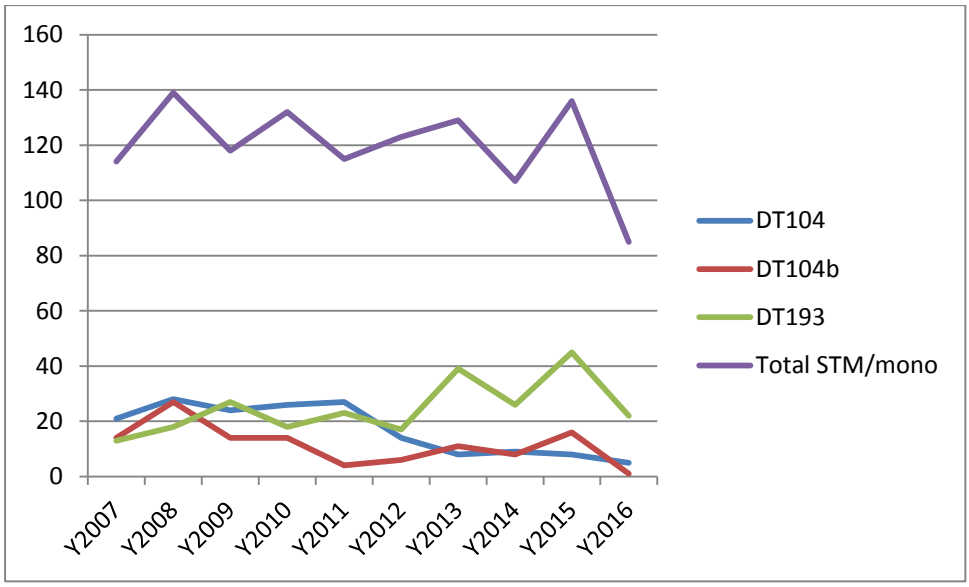
Poultry

A total of 22 isolates comprising 5 serovars were received from poultry sources. *S*. Enteritidis (n = 10) was the most common serovar received. *S*. Typhimurium and its monophasic variant 4,[5],12:i:- (n = 9) were also present. *S*. Enteritidis isolates were from a variety of poultry sources including duck and chicken. Isolates of these serovars were associated with human infections (*S*. Enteritidis (n = 83), *S*. Typhimurium/ monophasic Typhimurium (n = 85) in 2016.

Bovine

A total of 10 isolates were received from bovine sources and again *S*. Typhimurium and its monophasic variant 4,[5],12:i:- accounted for the majority of isolates (n = 6) with DT193 (n = 3) being the most common phage type. This phage type was also received from human cases of salmonellosis in 2016.

Figure 2 Fall in *Salmonella* Typhimurium DT104/DT104b isolates among isolates from swine



The fall in the number of isolates of *S*. Typhimurium DT104/DT104b in humans appears to correspond in time with a decline in the proportion of *S*. Typhimurium from pigs accounted for by these phage types. This may support the view that pigs/pig products have been an important source of domestically acquired infection with these phage types of *S*. Typhimurium in Ireland.

Antimicrobial Resistance among non-Human isolates

Antimicrobial susceptibility testing was performed on 14 of the 68 non-human isolates (54 were referred for phage typing only). Of the isolates tested 42.9% (n = 6) were susceptible to all antimicrobial agents tested while 28.6% (n = 4) 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

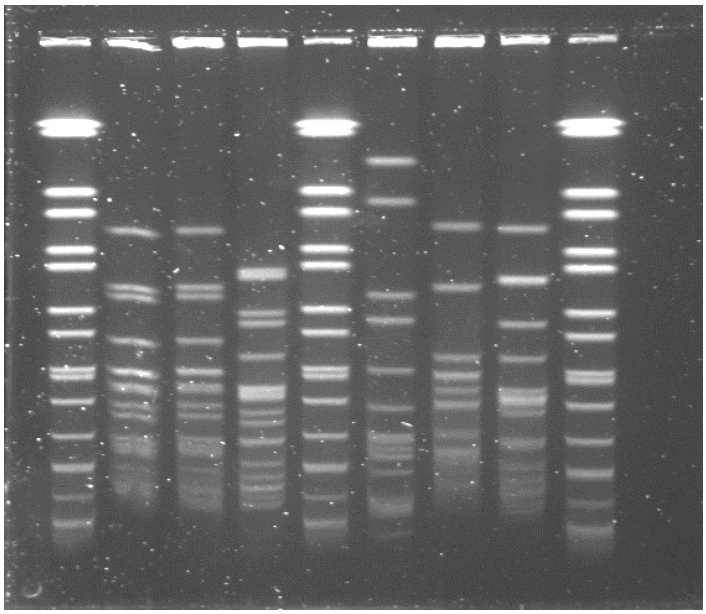
Table 5: Number and serotypes of *Listeria monocytogenes* isolates from human sources received in NSSLRL

Year	Total	4b	1/2a	1/2b	1/2c
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 9 *Listeria monocytogenes* isolates from human clinical samples in 2016. These included 5 from blood cultures, 2 from CSF, 1 from ascitic fluid and 1 from pus. Four of the isolates from humans typed as serotype 4b, 4 typed as serotype 1/2a and 1 typed as serotype 1/2b.

Fig. 3 Pulse Field Gel Electrophoresis of *L.monocytogenes* isolates digested with *Apa*I



Note: From 2017 PFGE date will not be available as the method has now been retired.

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*
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 4 Summary of *Shigella* isolates typed in NSSLRL from 2005-16

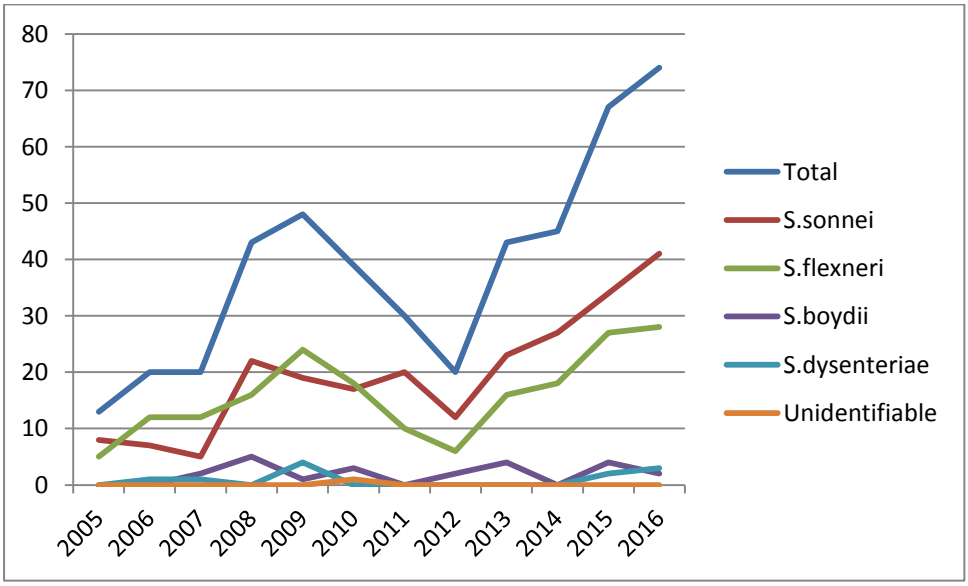
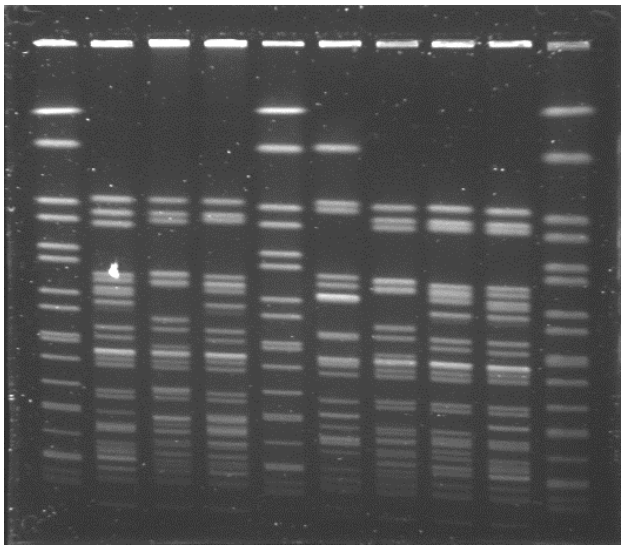


Fig. 5 Pulse Field Gel Electrophoresis image of *S.sonnei* digested with *Xba*I



Note: From 2017 PFGE date will not be available as the method has now been retired.

A total of 95 isolates were referred to the NSSLRL in 2016 for *Shigella* typing. When non-*Shigella*, QC and duplicate isolates were removed a total of 74 *Shigella* isolates and 1 enteroinvasive *E.coli* were typed. These consisted of 41 *S.sonnei* , 28 *S.flexneri*, 3 *S.dysenteriae* and 2 *S.boydii*. The *S.flexneri* isolates were further divided into 1 *S.flexneri* 1a, 2 *S.flexneri* 1c, 19 *S.flexneri* 2a, 1 *S.flexneri* 3a, 1 *S.flexneri* 3b, 2 *S.flexneri* 4c, 1 *S.flexneri* 6 and 1 *S.flexneri* X variant. This is the highest number of *Shigella species* ever submitted to the NSSLRL. It is worth noting that laboratories that have changed to use of direct molecular detection of pathogens in faces appear to have 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.

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, 82.5 %, of isolates (n = 61) were multi-drug resistant (three or more different classes of antibiotics). One ESBL producing *Shigella sonnei* (country of infection Ireland) was received in the NSSLRL in 2016 while 17 isolates were resistant to ciprofloxacin. The ciprofloxacin resistant isolates included 7 *Shigella sonnei*, 4 of which were known to be associated with travel to the Indian subcontinent, and 10 *S.flexneri* isolates, 3 with history of recent foreign travel to the Indian subcontinent. 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). The

NSSLRL added azithromycin to its antibiotic panel in October 2013 and in 2016 6/74 isolates exhibited resistance to azithromycin. These included 4 *S.sonnei*, 1 *S.flexneri* 1c and 1 *S.flexneri* 2a.

Thirty two patients had a recorded history of recent foreign travel, including Europe (n = 1), Africa (n = 9) and Australasia (n = 17). Twenty-eight patients had Ireland recorded as their country of infection while there were no details for 15 patients.

Table 7: Foreign travel history for *Shigella* isolates

Continent	Country	Number
Africa (n = 9)		
	Egypt	1
	Ethiopia	1
	Ghana	1
	Kenya	1
	Morocco	1
	Senegal	1
	South Africa	1
	Uganda	1
	West Africa	1
Europe (n = 1)		
	Europe	1
Australasia (n = 17)		
	India	7
	Bangladesh	2
	Pakistan	2
	Azerbaijan	1
	Indonesia	1
	Phillipines	1
	Thailand	1
	U.A.E.	1
	Vietnam	1
Americas (n = 4)		
	Brazil	2
	Colombia	1

Cuba	1
Foreign travel with country unknown	1

A large outbreak amongst men who have sex with men in the East of the country involving numerous *Shigella* serotypes and antibiograms which began in 2015 continued into 2016. These included *S*. *flexneri* 2a ACTTmNaCp (n = 4), *Shigella sonnei* SSuTTmNa & ASSuTTmNaAzt (n = 5), *S*. *flexneri* 2a ACST (n = 1) and *S*. *flexneri* 2a ACSSuTTm (n = 3). That outbreak is now closed.

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, e.g. serotyping, phage typing, and genotypic, e.g. PFGE, MLVA, in the subtyping of bacterial pathogens. Reference laboratories in some countries, e.g. Public Health England, Colindale have completely replaced conventional typing techniques with analysis of WGS, while other countries, e.g. CDC in USA, are in the process of changing to WGS. The process involves extraction and quantification of bacterial DNA followed by massive parallel sequencing which results in massive amounts of short sequence reads. Bioinformatics analysis involves the use of computers to extract useful information from these sequence reads. In 2016 we ran whole geneome sequencing in parallel with established methods for most isolates and for 2017 we have replaced phage typing and almost all molecular methods formerly used in our reference laboratory with whole genome sequencing.

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 acceptable [http://pubmlst.org].

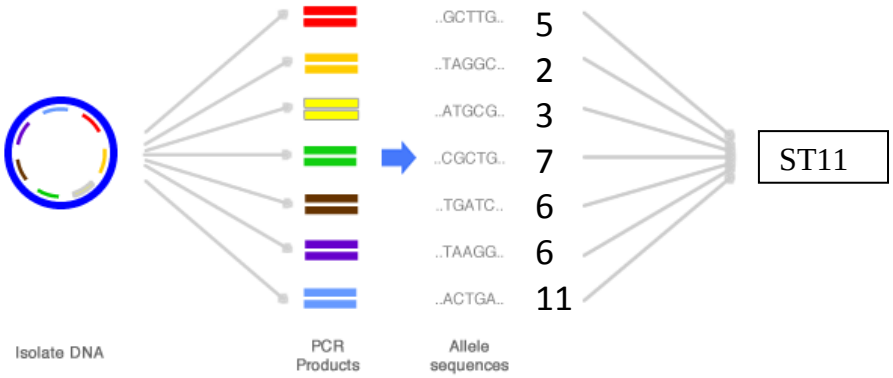


Fig. 6 Diagramatic representation of Multi Locus Sequence Typing (MLST) of *S*.Enteritidis.

'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...

Fig. 7 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 = 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.

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 is used.

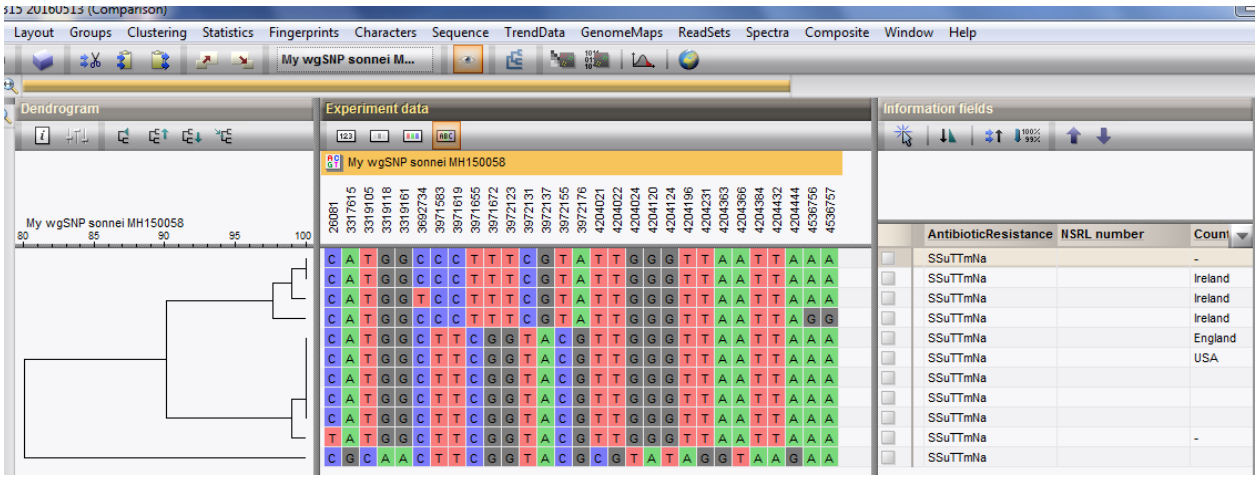


Fig. 7 Dendrogram of hqSNP analysis of *Shigella sonnei* using BioNumerics software.

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 will be acquired in 2017 with a view to in house sequencing in 2018.

DNA was extracted from all *Listeria* isolates received from human sources in the NSSLRL from 2010-15 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).

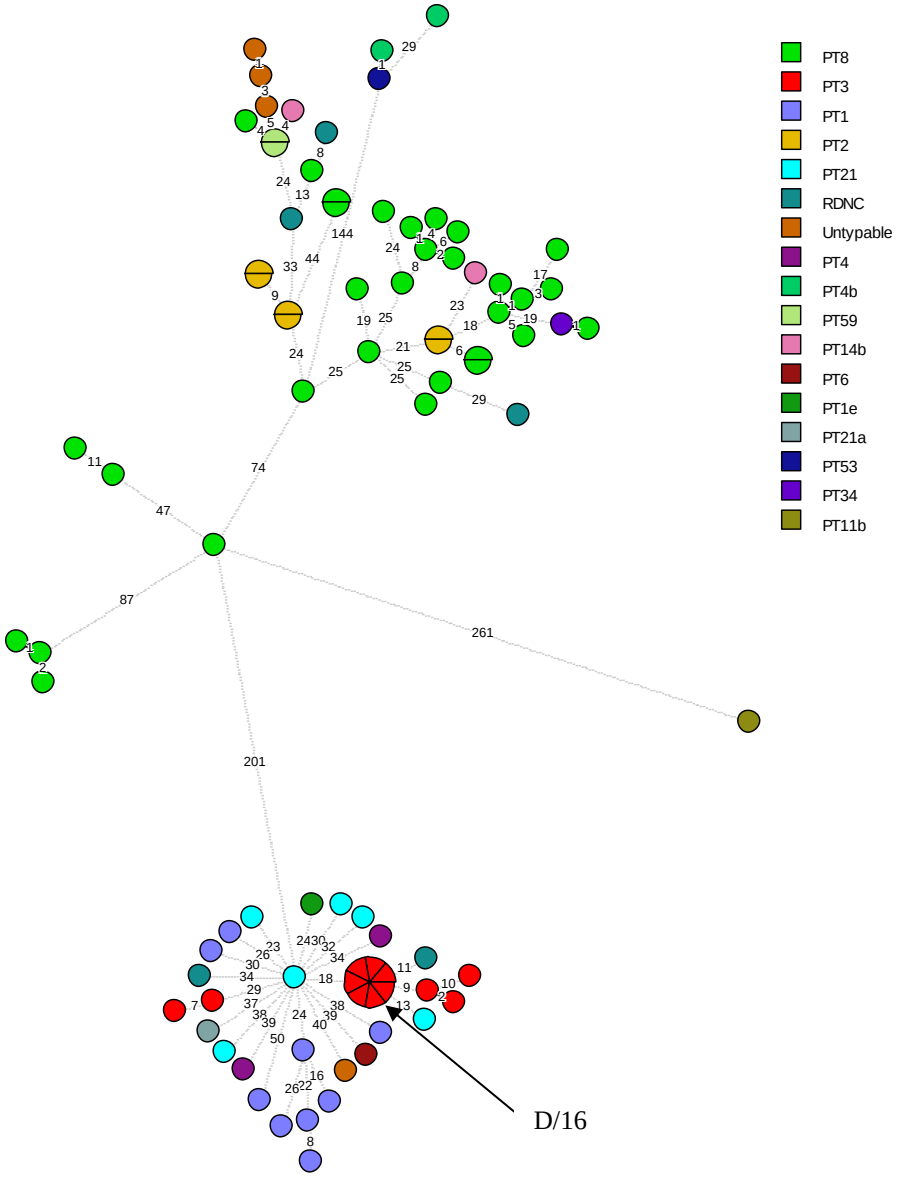


Fig. 8 Minimum spanning tree (MST) of *Salmonella* Enteritidis cgMLST from isolates from humans in the NSSLRL from 2015-16 coloured by phage type.

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. All the isolates within previously described *S*.Enteritidis clusters had identical cgMLST profiles. cgMLST provided much more discrimination that that provided by phage typing and MLVA analysis.

S.Enteritidis isolates were divided into two main lineages, one containing mainly PT8 and PT2 while the other contained PT1, PT21 and PT3. Some phage types, e.g. PT4 and PT14b, appeared in both lineages. The isolates from the foods associated with family outbreak “D/16” were identical to

the isolates from members of the family by cgMLST. [The code D/16 is NSSLRL designation for 4th such cluster identified in the year 2016]

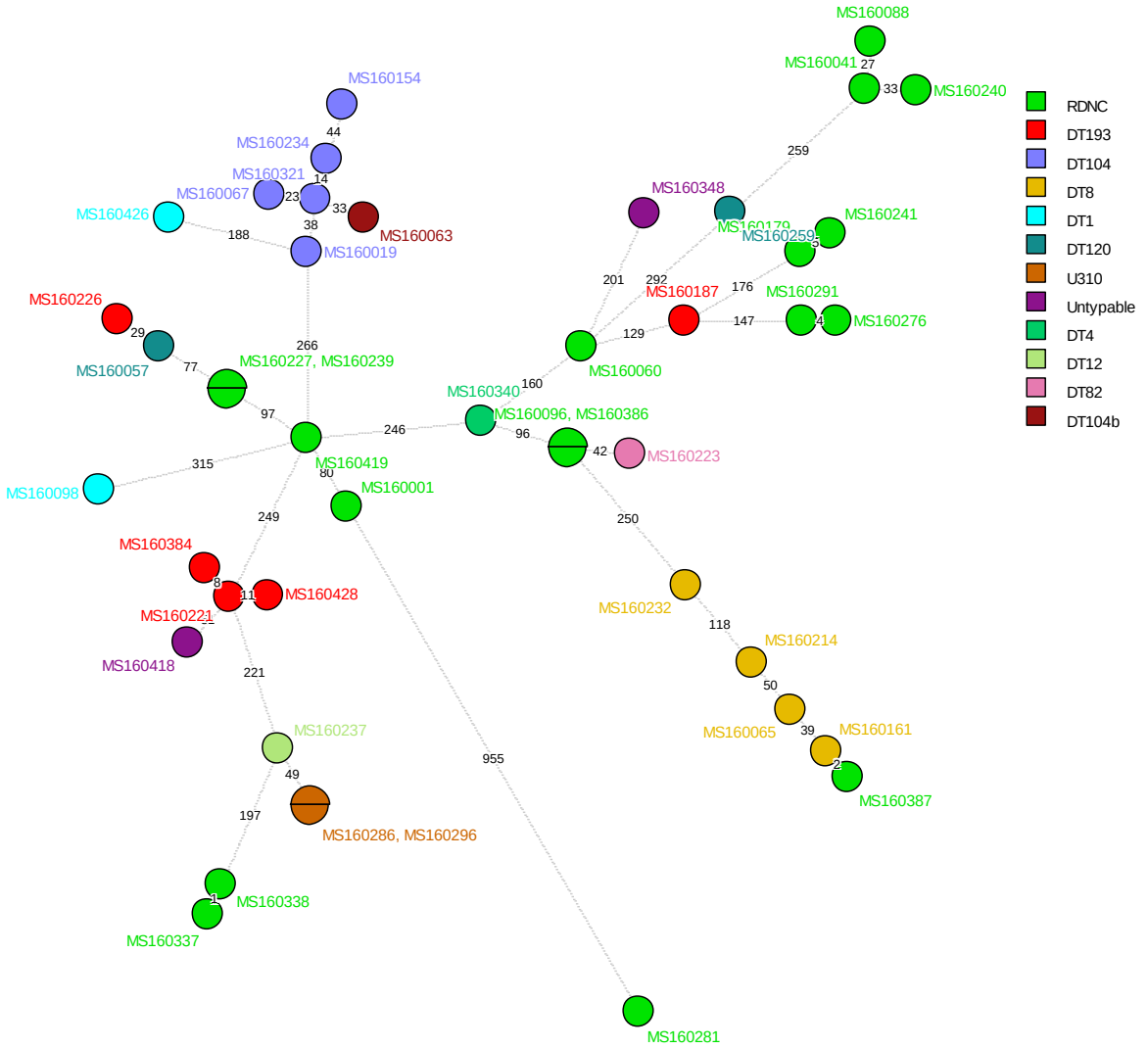


Fig. 9 cgMLST of ST19 (*S*.Typhimurium) isolates coloured by phage type. All the previously described clusters had identical or very closely related cgMLST profiles.

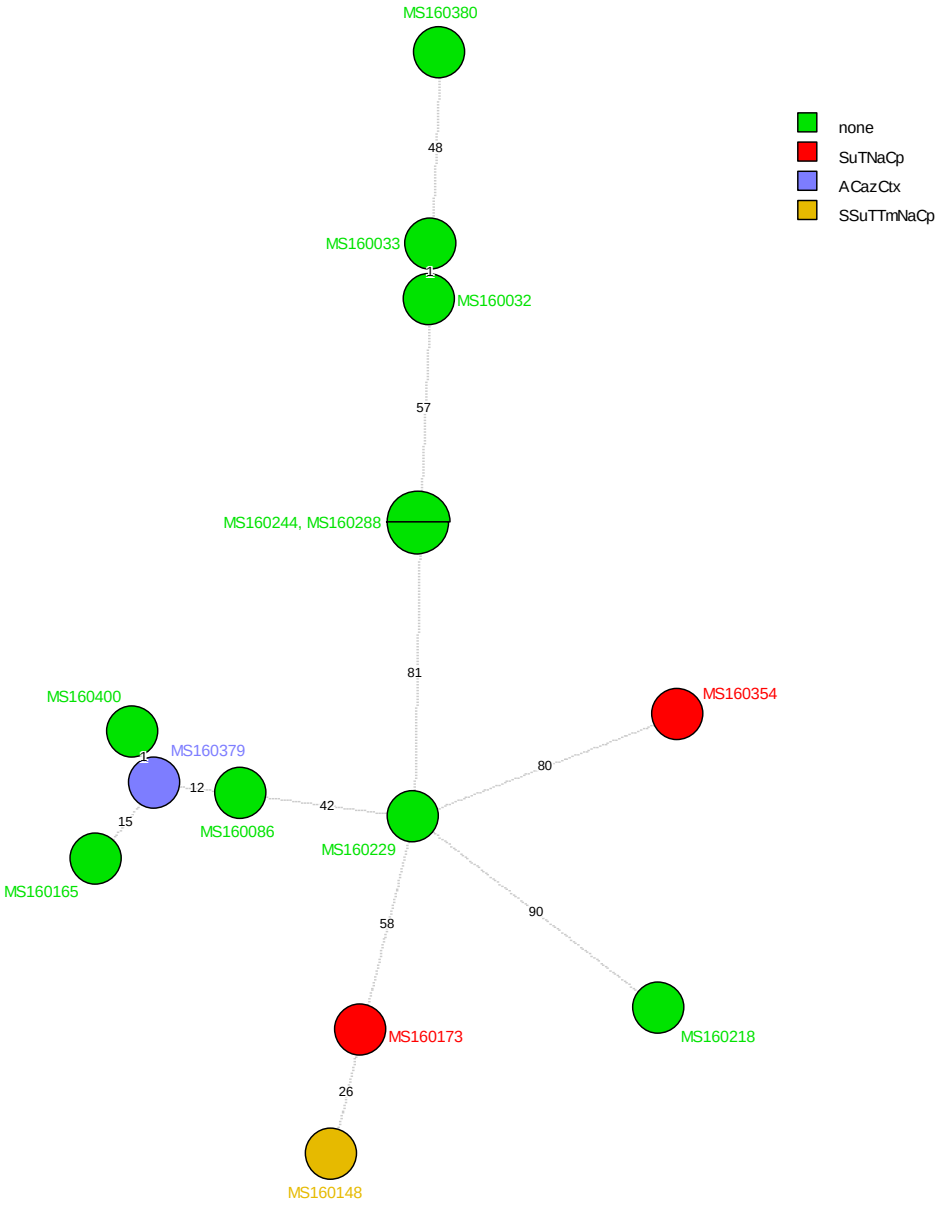


Fig. 10 cgMLST of *S*.Infantis isolates divided by antibiogram. A total of 14 Infantis isolates, 12 from humans, were analysed. Indistinguishable isolates MS160244 and MS160288 were from water. A/16 (MS160032 and MS160033) was a family outbreak associated with travel to Spain. MS160379 and MS160400 had only 1 allele difference even though one was fully susceptible and the other was an AmpC producer. Both had travel to Spain.

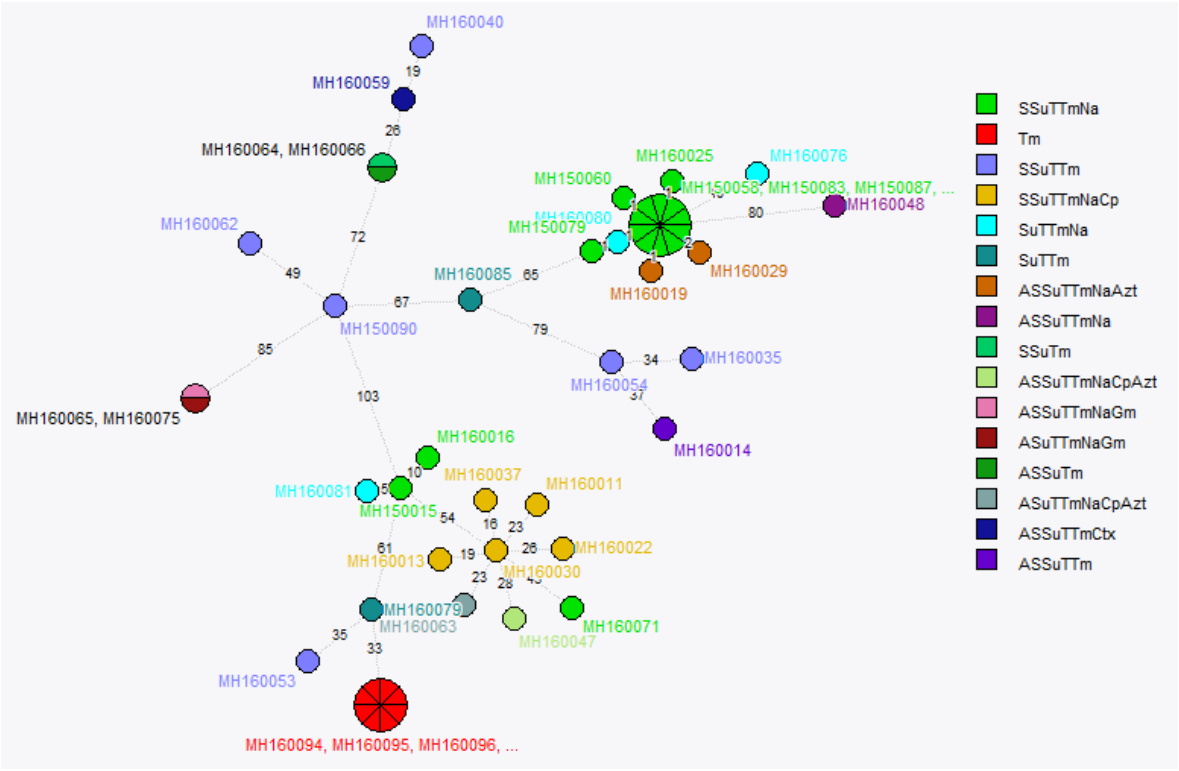


Fig. 11 cgMLST of *Shigella sonnei* isolates from humans in the NSSLRL from 2015-16 divided by antibiogram. The *sonnei* isolates had 12 different antibiograms. Testing of streptomycin was discontinued from 1st October 2016, therefore in each of the pairs below an isolate with the antibiogram given first is indistinguishable from the antibiogram given second. SSuTTmNa/SuTTmNa, SSuTm/SuTm, SSuTTm/SuTTm, ASSuTTmNaCpAzt/ASuTTmNaCpAzt, and ASSuTTmNaGm/ASuTTmNaGm.

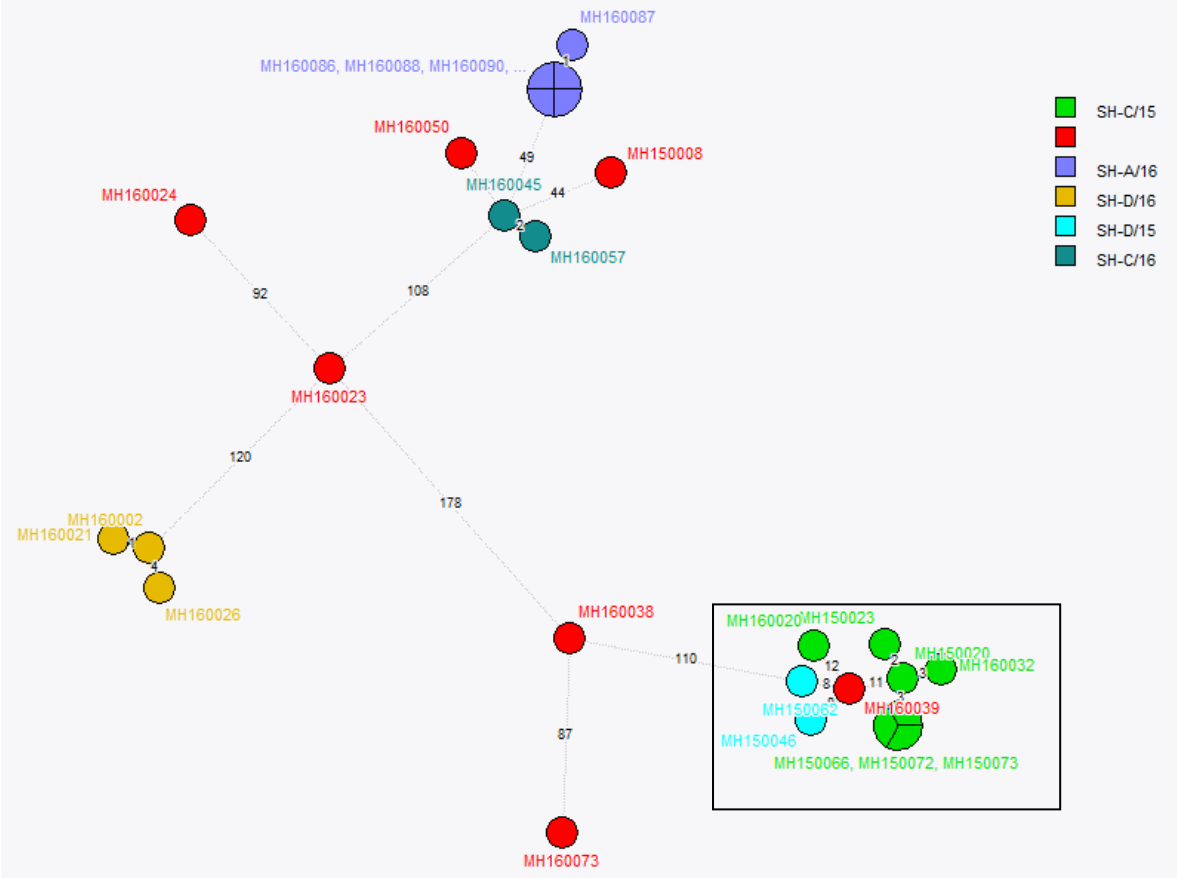


Fig. 12 cgMLST of *Shigella flexneri* 2a isolates from humans in the NSSLRL from 2015-16 grouped by cluster.

Cluster SH-C/15 was an outbreak in the Dublin area among men who have sex with men and all isolates had the same antibiogram, ACST. Cluster SH-D/15 consisted of two isolates with same antibiogram, ACSSuTTmAzt, with primary sample dates about 2 weeks apart, both from adult males, one of whom identifie as MSM. SH-A/16 was a cluster of 4 isolates with the same rare antibiogram, ACTTmNaCp, all from adult males in the Dublin area from late 2016. All were MSM and there was an epidemiological link to a specific MSM venue. There was <= 1 allelic difference between the isolates. SH-D/16 was a cluster involving 3 males in their 50s
from the East with the same antibiogram, ACSSuTTm, and with primary sample dates from early 2016 (Jan, Feb and Apr). One man identified himself as MSM.

NSSLRL Publications and Presentations 2016

Talks/Poster Presentations

Comparison of Centre for Genomic Epidemiology and Public Health England Pipelines for Analysis of Short Read Sets. I3S, St Malo, France. June 6-8th. 2016.

Comparison of PulseNet (CDC) and ECDC MLVA Typing Methods for *Salmonella* Enteritidis: A 3 year retrospective study. I3S, St Malo, France. June 6-8th. 2016.

"NSSLRL Experience of WGS" and "Comparison of wgMLST and hqSNP Analysis". A Discussion on Methods and Challenges of using WGS for surveillance of Food-Borne Pathogens. SSI, Copenhagen. Oct 6-7th. 2016.

Papers/Letters

South Asia as a reservoir for the global spread of ciprofloxacin resistant *Shigella sonnei*. A cross sectional study. PLOS Medicine doi:10.371/Journal.pmed.1002055.