Galway Reference Laboratory Service Users Guide

(incorporating the National *Salmonella, Shigella* & *Listeria* (NSSLRL) and National Carbapenemase Producing Enterobacterales (CPE) Reference Laboratories)

1.0 Role of the Reference Laboratory and Outline of Services

The Galway Reference Laboratory Service are based in the Department of Medical Microbiology at GUH and are offered to all medical laboratories in hospitals throughout Ireland.

The Department of Medical Microbiology at GUH participates in available external quality assurance schemes and is accredited by the Irish National Accreditation Board (INAB) to undertake testing as detailed in the Schedule bearing the Registration Number 223MT in compliance with the International Standard ISO/IEC 15189:2022.

Role of NSSLRL

1. Support clinical laboratories by confirming and subtyping *Salmonella*, *Shigella* and *Listeria* isolates.
2. Recognise links between individual cases of infection, even where outbreaks are widely dispersed. To provide support in the investigation of suspected outbreaks.
3. Provide national data to inform public health on the scope issue/outbreak and the effectiveness of responses.
4. Liaise with HPSC and the European Centre for Disease Control (ECDC) to help identify and control international dimensions to outbreaks. This involves creating alerts for Ireland and analysing other countries outbreak alerts. This involves sharing sequencing data without any patient identifying data.
5. Liaise with the Department of Agriculture and the Marine (DAFM) to help identify and control national outbreaks. This involves sharing sequencing data without any patient identifying data.

This information is primarily to guide public health intervention to recognise and control transmission of infection.

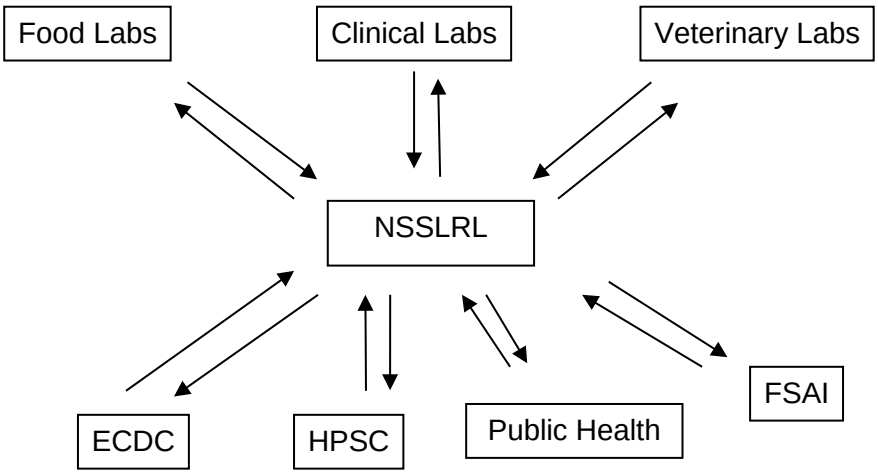

Fig.1 NSSLRL Organisational Chart

The NSSLRL works in tandem with numerous agencies in protecting public health. The laboratory which identifies the pathogen and the clinician who is responsible for care of the patient are both obliged to notify the case to Public Health (PH). Public Health services in the Health Regions and at National level lead on and coordinate multidisciplinary investigation and management of incidents and outbreaks. Other partners in incident and outbreak management include the Environmental Health Service, the Food Safety Authority of Ireland, the Department of Agriculture Food and the Marine and ECDC.

Stool specimens may subsequently be submitted to clinical laboratories to determine if contacts of the cases are also infected or colonised. If a relevant pathogen is isolated in those laboratories, the isolates may be sent to the NSSLRL for comparison with the isolate from the first case or outbreak. Food (and occasionally water) samples may be sent to a food and water laboratory to try to determine a source of infection. Isolates from those samples are submitted to the DAFM Reference Laboratory for characterisation. Data is shared between reference laboratories to assist in identifying links between food and human isolates.

As part of an incident or outbreak investigation a questionnaire may be administered to investigate possible risk-factors (contact with animals, occupation, etc.), travel history and recent food history. When more than one case is detected cross-comparison of questionnaires may allow a putative source to be identified.

Role of the National CPE Reference Laboratory

1. Support clinical laboratories by differentiating between carbapenem resistance due to mobile genetic elements and carbapenem resistance due to other reasons. And to confirm and subtype the detected CPE gene.
2. Aid in tracing pathways of spread of CPE by assessing the degree of similarity between CPE and between certain plasmids carrying carbapenemase genes (pOXA48 and pOXA181) from different patients and from different hospitals
3. Support investigation of suspected outbreaks of CPE infection/colonisation.
4. Provide national data to inform HSE AMRIC and public health on the scope of the problem and the effectiveness of responses
5. Liaise with HPSC and the European Centre for Disease Control (ECDC) to help identify and control international dimensions to outbreaks. This involves sharing data, countries issuing outbreak alerts.
6. Provide extended antibiotic susceptibility testing on clinical CPE when requested.

Note: The microbroth dilution plates used by the reference laboratory only contain Meropenem, Piperacillin-Tazobactam, Ceftazidime-Avibactam, Ceftozalone-Tazobactam and Colistin. No other antimicrobial agents are available.

The CPE reference laboratory provides a clinically supported service for the detection of carbapenemase producing Enterobacterales. other Carbapenemase Producing Organisms (CPO) including *Acinetobacter spp.* and *Pseudomonas aeruginosa* were not part of the service the laboratory was established to deliver. However, as these have emerged as a significant concern isolates of these general are characterised in so far as possible in response to concerns regarding meropenem resistance in these genus. The laboratory also seeks to support clinical laboratories in the investigation of incidents involving Gram-negative bacteria other than CPE where there is no other reference laboratory providing the required service and the laboratory is able to manage the associated workload. Requests for such support should be made by the director or the referring laboratory to the director of the Galway Reference Laboratory Services.

This guidance for users represents an attempt to target the available resources to deliver the service so as to maximize detection of the major CPE concerns. Given the variety of known CPE enzymes, variability of gene expression, other mechanisms that result in raised carbapenem MIC and the rapidly changing epidemiology it is acknowledged that no set of selection criteria for isolate submission can ensure that every CPE producing isolate is confirmed. The CPE reference laboratory service would like to acknowledge helpful comments and calls from colleagues in laboratories around the country highlighting specific concerns which have been considered in revising this guidance and would appreciate if you would continue to alert the service to concerns and emerging issues.

2.0 Laboratory Policy

The Division of Clinical Microbiology is committed to providing a timely, efficient and quality diagnostic and reference laboratory service to all patients, clinicians and other users of the service. Laboratory Management is committed to maintaining and continually improving the quality system so that the requirements of ISO15189:2022 and the Irish National Accreditation Board (INAB) are met on an ongoing basis. Antimicrobial susceptibility testing is an accredited test, however, WGS remains a non-accredited test. Although most Reference Laboratory Services are not on the scope of accreditation of the laboratory the quality systems that apply to services are similar to those that apply to services on the scope of accreditation.

It is Division policy that all staff are trained and have familiarised themselves with the quality documentation to ensure they can implement all policies and procedures. The Division is committed to good professional practice and to the provision of examinations that are fit for intended use to achieve a quality of service that is compliant with their quality management system and therefore the international standard ISO 15189.

3.0 User information

Service Level Agreement (SLA)

The request form for the NSSLRL or CPE Reference Laboratory serves as the formal 'Service Level Agreement' between the Galway Reference Laboratory Services, Diagnostics Directorate, Galway University Hospital (GUH) and the Service user.

Contact Details

This user guide is designed to assist the client in the use of the services we offer. Further information is available by telephoning the laboratory.

Director: Prof. M.Cormican (091) 544146 martin.cormican@hse.ie

Microbiology Consultants:

Dr.Deirbhile Keady at deirbhile.keady@hse.ie , Dr.Una NiRiain at una.niriain@hse.ie , Dr.Teck Wee Boo at teck.boo@hse.ie , Dr.Ruth Waldron at ruth.waldron@hse.ie , Dr.Dimitar Nashev at Dimitar.Nashev@hse.ie, Dr.Roisin Mulqueen at roisin.mulqueen3@hse.ie

Address: Galway Reference Laboratory Service (NSSLRL & CPERL)
Department of Medical Microbiology
University Hospital Galway
Galway
H91 YR71
Laboratory
Tel: (091) 544628
Fax: (091) 542238

e-mail: Christina.clarke@hse.ie or Alma.tuohy@hse.ie

Opening Times: Monday to Friday 9.00am - 5.00pm

Out of hours work

This is performed at the discretion of the Consultant Microbiologist or the laboratory scientific staff on urgent samples, e.g. during an outbreak investigation.

Laboratory Contact Out of Hours: (091) 544411

Confidentiality Policy

It is the responsibility of all staff, as defined in their contract of employment, to ensure that all information which they have access to as part of their work is treated in the strictest confidence and protected from unauthorised access. All staff are asked to sign a confidentiality agreement during their laboratory induction programme.

4.0 Services Provided

The Reference Laboratory only performs analysis on isolates received on nutrient agar slopes. The laboratory does not test primary samples, e.g. swabs, faeces, blood or food, therefore results are qualitative and not quantitative. The laboratory does not accept isolates from food or animals as these should be directed to the relevant Reference Laboratory Service.

If users wish to use data from the Reference laboratory in publications they must contact the laboratory director at martin.cormican@hse.ie or in his absence one of the scientific staff.

4.1 Isolates to be Referred for Analysis of Whole Genome Sequences (non-accredited)

National *Salmonella*, *Shigella* and *Listeria* Reference Laboratory Isolates:

- All *Salmonella species*.
- All *Shigella species*.
- All *Listeria species.*

National CPE Reference Laboratory Isolates:

- **All Diagnostic isolates with a CPE target detected,** e.g. by molecular or lateral flow. Diagnostic isolates includes isolates from blood, urine, wounds, sputum and other samples submitted for general diagnostic culture. It does not include samples submitted for routine testing for CPE colonisation in the absence of any clinical suspicion of infection.
- **Screening isolates (rectal/faeces etc.) with a CPE target detected,** e.g. by molecular or lateral flow.
    - **Large volume users with a quota-** Your laboratory has been informed by email if your laboratory has a limited quota for screening isolates and on how many isolates can be sent per quarter. In 2025 this applies to all Model 4 hospitals. This was introduced of necessity as of January 1st 2025 as the volume of isolates submitted from Model 4 hospitals in 2024 had grown to a unsustainable degree reflecting the continued growth in numbers of people acquiring CPE colonisation.
        - Please send representative isolates.
        - Please contact the laboratory if you are experiencing an outbreak or other situation that may require flexibility on the quota
    - **Users without a quota-** Please send all screening isolates with CPE target detected.

- **Isolates with CPE targets NOT detected meeting below criteria:**
    - **Enterobacterales for unusual CPE detection**
        - **Diagnostic Samples -** Enterobacterales with an MIC to Meropenem of $\geq$ 2 mg/L (isolates with an MIC of 1 or lower do not need to be submitted).

- **Screening Samples** – Enterobacterales with a Meropenem MIC > 8 mg/L.

- ***Klebsiella pneumoniae* isolates for Hypervirulence**

  Testing from diagnostic speimens only.

- ***Acinetobacter species***

  Any high (Meropenem MIC > 8 mg/L) or unusual resistance with the caveat that the National Reference Laboratory Service would not have the resources to process all such *Acinetobacter spp* isolates and the yield of CPE would be very low. However if Clinical Laboratories are concerned about specific strains particularly if there appear to be 2 or more linked patients or recent hospitalization outside of Europe the reference laboratory will accept the isolates by prior arrangement.

- ***Pseudomonas species***

  Generally these should only be submitted if they show exceptional levels of resistance or have a carbapenemase antigen or gene detected. However, if Clinical Laboratories are concerned about specific strains particularly if there appear to be 2 or more linked patients or recent hospitalization outside of Europe the reference laboratory will accept the isolates by prior arrangement.

  Note:

  ***Proteus* spp.** and ***Morganella* spp**. are intrinsically resistant to Imipenem.

  ***Enterobacter* spp.** resistant to cephalosporins and with low level resistance to ertapenem but susceptible to meropenem typically represents the combination of AmpC and impermeability rather than carbapenemase production.

  Stenotrophomonas maltophilia is intrinsically resistant to carbapenems.

Environmental screening for CPE:

If environmental screening for CPE is performed and you wish isolates to be typed you may used the assigned quota for either screening or environmental isolates. If you do not have an assigned quota please email an excel sheet of the isolates listing species, CPE gene, source and any other relevant information to the reference laboratory @ Christina.clarke@hse.ie or Alma.tuohy@hse.ie. This list will be discussed with the laboratory director, Prof. Cormican, and you will be contacted stating which isolates to refer. Note tha

Unusual organisms outbreaks:

Please contact the laboratory to discuss, and where possible typing may be performed

4.2 Request Form & Labelling Requirements

The NSSLRL or CPE request form must accompany and identify all isolates. Currently the request forms are available to download on the Saolta (www.saolta.ie) website using the search function.

Please note if the request form/slope is handwritten it must be clearly legible.

The **request form** must contain the following information:

- Patients Name
- Patients Date of Birth
- Referring laboratory name
- Referring laboratory contact details **including a direct telephone number for the laboratory and a secure email address that can be used to communicate regarding patient results**.
- Laboratories isolate reference number
- Primary sample date (NSSLRL only)
- Meropenem MIC (CPERL only)
- Carbapenemase detection kit result (if available) (CPERL only)

The referring **slope** must contain the following information:

- Patients Name **and**
- Date of Birth Or hospital number (hospital number must be on the request form if using this as an identifier)
- Laboratories isolate reference number

Ninety-five percent of samples will be reported within 28 working days and specimens that are identified by telephone call as urgent will be prioritised.

4.3 Tests Performed

***Salmonella, Shigella* and *Listeria* isolates**

- Whole genome sequencing is performed.

Query Carbapenemase producing isolates:

- Identification by MALDI-TOF MS

- Note: in some instances where the MALDI-TOF MS can only identify an isolate to species level the data generated from Whole Genome Sequencing may be used to further identify the isolate e.g. *Citrobacter species* can be further identified using 'Species Identification by Ribosomal MLST' or using Mash Distance.

- Whole Genome Sequencing (WGS) on carbapenemase producing Enterobacterales and other carbapenamase producing isolates, e.g. *Acinetobacter*, see Appendix 9.2.
- Colistin MIC and ceftazidime-avibactam MIC (where appropriate) by broth dilution is routinely performed on confirmed carbapenemase producing Enterobacterales isolated from clinical samples. Limited additional susceptibility results are available on request.

4.4 Results and Turnaround Times

The reference laboratory aims to report 95% of results within 28 working days. The average time will be 15 working days following receipt. Results are transmitted to users by hard-copy. However, if necessary, unusual or important results may be provisionally reported either via phone or email directly to the relevant consultant microbiologist if contact details such as mobile phone or secure email address are provided.

At present the turnaround times for reports are generally well within the targets above however occasions may arise.

Please contact the laboratory if there is a requirement for urgent processing and we will try to prioritise the isolates.

5.0 Specimen Rejection Policy

Specimens sent to the reference laboratory will be not be processed under the following conditions:

- Unlabelled slope and/or request form
- Mislabelled slope and/or request form
- Broken slopes
- Mixed cultures

- Where the species stated on the request form does not correlate with the identification of the slope received.
- If isolates of the same phenotype/genotype are received from the same patient and site within 6 months of a previously sequenced isolate they will be rejected, e.g. *Salmonella* from stools, *E.coli* with *bla*OXA48 from a rectal swab. If the isolate(s) comes from the same referring laboratory it is rejected with a comment stating "A previous isolate was received from this patient within the last 6 months. Please see report ME… for WGS results."

  If the isolate is received from a different referring laboratory the result from the first isolate is entered along with a comment stating "This isolate was not sequenced. An isolate with this genotype was received from another hospital within the last 6 months. The sequencing results in this report are from that isolate."

6.0 Transportation of Specimens

- Inoculated slopes should be packaged and transported according to ADR (Carriage of Dangerous Goods by Road) regulations.
- A number of slopes may be sent in each container but each slope must be individually wrapped in an absorbent material to prevent breakage during transit.

7.0 Retention Times

- All slopes referred are stored for a minimum of 48 hours after analysis.
- Slopes that exceed assigned the assigned quota without prior agreement will be stored for a short period after issue of a report indicating the isolate was not processed.
- All referred isolates that are processed for typing are stored on PROTECT beads at -25ºC *(Salmonella, Shigella & Listeria)* or -80°C (CPE) for a minimum of 3 years.
- All DNA extracts are stored at -20°C for a minimum of 1 year.

8.0 Complaints

Consumer Affairs and the National Advocacy Unit, Quality and Patient Safety Directorate have responsibility for developing and implementing best practice models of customer care

within the HSE and promotes service user involvement across the organisation through the concept of

"Your Service Your Say".

Note: If users have a complaint or feel that any part of the service is unsatisfactory or could be improved on in any way we invite you to contact the laboratory.

9.0 APPENDIX

9.1 Whole Genome Sequencing

Whole Genome Sequencing (WGS) is performed on IlluminaNextSeq1000 platform. Data analysis of sequenced data is performed using commercial software – SeqSphere+ (Ridom) which is supplemented where necessary with additional available software e.g. PubMLST and CGE's Resfinder (Centre for Genomic Epidemiology).

These are regularly updated so to avoid sending multiple memos and updates of user guides to referring laboratories a list of the version numbers and revisions is maintained in the reference laboratory and is available on request.

9.1.1 Reporting WGS Data

The hard copy report issued by the reference will include the following WGS results:

Sequence Type

A Sequence Type (ST) will be reported where a MLST scheme is available. Sequence Type is based on allelic variation in designated housekeeping genes, normally seven, of a particular species. Variations in allele sequences can arise by single point mutations, insertions and/or deletions or indels and inversions. For example *Salmonella* Enteritidis has MLST profile 5,2,3,7,6,6,11 which has been assigned ST11 while S.Typhimurium has MLST profile 10,7,12,9,5,9,2 which corresponds to ST19. If two or more isolates are of different ST it can be taken **t**hey are non related strains. If they are the same ST further discrimination can be provided by comparisons of core genome (cg) or pan genome (pg) MLST (see below). Occasionally, for further discrimination, we may look at the accessory genome.

Resistance Determinants

Salmonella and Shigella

All genes encoding resistance to the following antibiotics; ampicillin, chloramphenicol, sulphamethoxazole, tetracycline, trimethoprim, nalidixic acid, ciprofloxacin, gentamicin, ceftazadime, cefotaxime and azithromycin. Mutations in the *gyrA*, *gyrB* and *parC* genes resulting in increased ciprofloxacin MICs are also reported.

Resistance Genes

CPE

We currently report on the presence of:

- Carbapenemase genes
- ESBL genes
- AmpC genes
- Other b-lactamases
- presence/absence of *mcr* gene (mobile colistin resistance gene)
  although note that some *mcr* genes (for example *mcr*.9) are not clearly associated with a phenotype of colistin resistance.
- For *K.pneumoniae* a hypervirulence score is assigned

Intrinsic Resistance genes

Some bacterial species encode intrinsic resistance genes, i.e. always expressed in a species, independent of previous antibiotic exposure and not related to horizontal gene transfer.

Examples of intrinsic beta lactamase/carbapenamase include …

- *Klebsiella oxytoca* encode *bla*OXY genes
- *Raoultella species* have intrinsic *bla*PLA-1 (*R.planticola*), *bla*ORN-1 (*R.ornitholytica*) and *bla*TER-1 (*R.terrigena*)
- *Klebsiella pneumonia blaSHV-1*
- *Serratia* species and SRT-2
- *Acinetobacter radioresistens* has a chromosomally encoded *bla*OXA-23 gene

- *Acinetobacter baumanii complex* has a chromosomally encoded *bla*OXA-51 gene
- *Pseudomonas aeruginosa* has intrinsic resistance genes *bla*PAO and *bla*OXA-50

These intrinsic genes are not reported.

Plasmids

We currently report:

- IncL/M (pOXA-48) – plasmid associated with spread of OXA-48 carbapenemase gene
- IncX3 – plasmid associated with spread of OXA-181 carbapenemase gene

Species identification

The vast majority of isolates that arrive in the CPE reference laboratory have already been identified by the sending laboratory. For isolates referred to the CPE laboratory identification is confirmed by MALDI-TOF MS which is sufficient for the majority of bacterial species. However MALDI-TOF MS is unable to speciate *Citrobacter* isolates and also has difficulties distinguishing between *Klebsiella oxytoca* and *Raoultella* species.

Ribosomal multi locus sequence typing (rMLST) at https://pubmlst.org/rmlst/ looks at variation of the 53 genes encoding the bacterial ribosome protein subunits (rps genes). These genes are ideal as a universal typing scheme as (i) they are present in all bacteria, (ii) they are distributed around the chromosome and (iii) they encode proteins that are under stabilizing selection for functional conservation. A combination of these two methods was used in a validation in this laboratory. Following this validation identification of the vast majority of referred isolates will still be based on the MALDI-TOF MS reading while rMLST will be used in a limited number of cases.

Mash Distance is a software for fast genome distance estimation using the MinHash algorithm. In SeqSphere+ Mash Distance is used for rapid species identification and automatic project choosing in the pipeline. For this purpose, SeqSphere+ comes with a Mash reference database (sketch size of s=1,000 and k=21) that contains all prokaryotic NCBI Genome entries with status complete or chromosome that were filtered for taxonomic reliable genus and species information (11,646 genomes, as of February 2019).

Klebsiella oxytoca/ Raoultella species

Isolates will be reported as *K.oxytoca* if they are identified as such by MALDI-TOF and contain a *bla*OXY gene, otherwise identification will be based on rMLST. Where WGS has not been performed isolates called *K.oxytoca*/*Raoutella* by MALDI-TOF MS will be differentiated based on the majority MALDI-TOF MS call. Suspected *Raoultella* isolates will be identified and reported to species level by rMLST.

Citrobacter species

Citrobacter species can be divided into groups

A: (*C.freundii complex*) including *C.freundii, C.braakii, C.youngae, C.murilinae, C.werkmanii, C.gillenii, C.rodenticum, C.sedlakii*, *C.europaeus* and *C.pasteurii*

B: *C.amalonaticus* and *C.farmeri*

C: *C.koseri/diversus*. Many of the *Citrobacter* species within the *C.freundii* complex are closely related and difficult to distinguish between.

Mash Distance is performed on all *Citrobacter* sequences and reported as either *Citobacter freundii* complex, *C.amalonaticus* complex or *C.koseri*

Acinetobacter

The MALDI-TOF MS identification will be accepted for isolates belong to the *Acinetobacter baumannii* complex and a ST will be reported if present.

Serratia

Mash Distance and or rMLST will be performed on *Serratia* species

Klebsiella variicola

Klebsiella variicola are analysed using the SeqSphere+ software *K.pneumoniae* scheme and sequence type will be reported if present.

Hypervirulent *Klebsiella pneumoniae*

Classic *K.pneumoniae* isolates primarily cause nosocomial infections in immunocompromised patients. Hypervirulent *K.pneumoniae* (hvKp) are associated with infection of previously healthy patients and cause metastatic infections including liver abscesses and blood stream infection. The virulence factors including siderophores such as salmochelin and aerobactin are encoded on mobile elements. hvKp were primarily associated

with the Pacific rim but in recent years have spread globally. All *K.pneumoniae* isolates sequenced in the reference laboratory are assessed for genes encoding these factors and a score is assigned based on the Kleborate scoring system used by ECDC,

Kleborate scoring system

- 1 = Yersinibactin only
- 2 = Colibactin without Aerobactin
- 3 = Aerobactin only
- 4 = Aerobactin and Yersiniabactin
- 5 = Aerobactin, Yersiniabactin and Colibactin

Isolates of *K.pneumoniae* with a score of 3 or more are reported as hypervirulent *K.pneumoniae*.

9.1.2 Outbreak Investigations

Line listings will be sent out at regular intervals.

Cluster codes will be assigned to isolates related by cg/pgMLST. The code name includes the species, the year the cluster was discovered and the number of clusters of that species called for the year, e.g. EC22-001 indicates the first *E.coli* cluster called in 2022. Of note this may include isolate(s) from earlier years.

Identifying clusters can be difficult and should not be used in isolation to exclude transmission in the healthcare setting. Results should always be considered along with epidemiological data. Users are welcome to contact the laboratory to discuss results.

Where the reference laboratory identifies a cluster involving more than one site the reference laboratory may alert the sites involved.

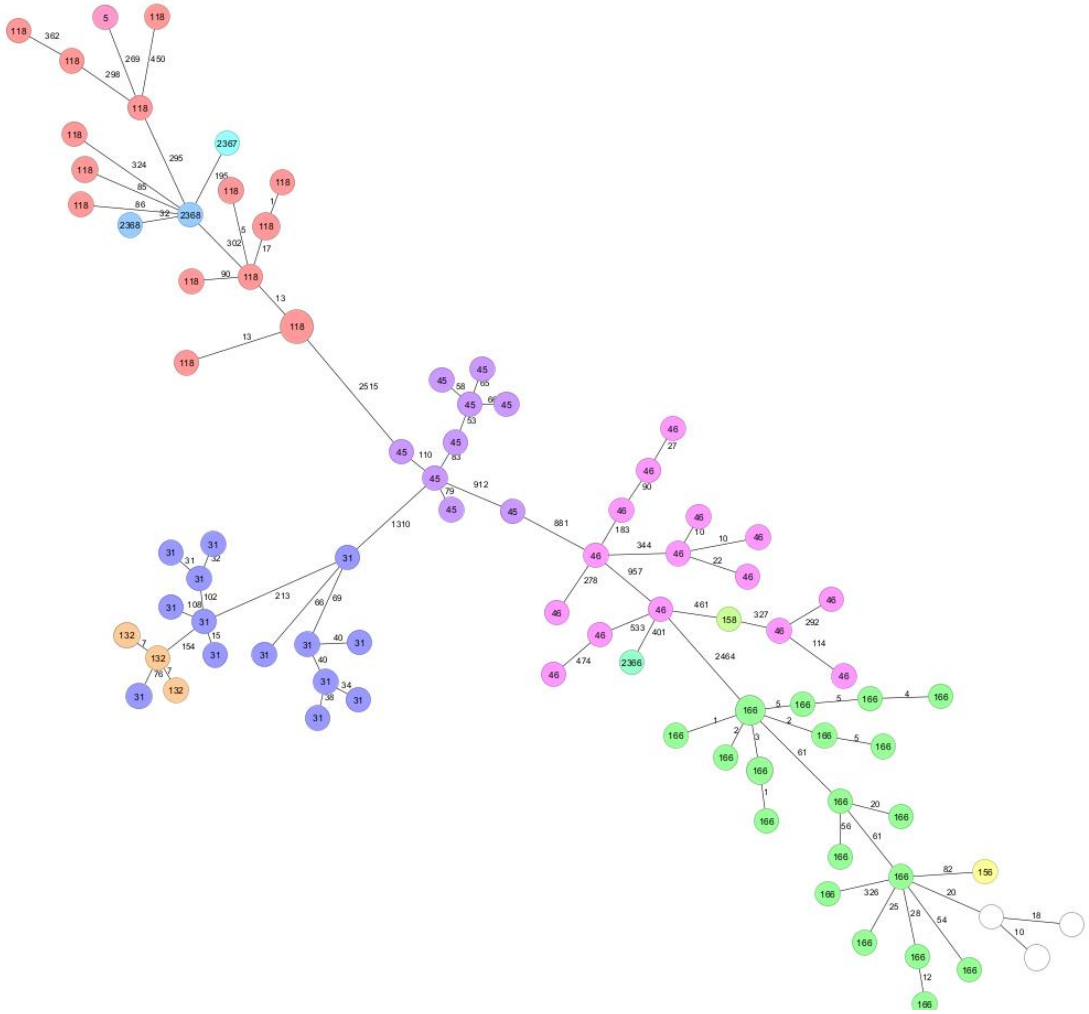

Fig.2 cgMLST of *S*.Newport isolates from 2017-19 coloured by Sequence Type Most *Salmonella* serotypes are generally monophyletic. Essentially this means that the antigenic formula that defines the serotype reflects a common genetic origin for members of the serotype. However, this is not true in all cases. In some cases organisms that are very different at a genetic level have converged on the same antigenic formula by acquisition of genes encoding for the same set of O and H antigens. These serotypes are considered polyphyletic.

For example, *S*.Newport (6,8:e,h:2) is a polyphyletic serotype, i.e. isolates with this shared antigenic structure have originated at different times on different genetic backgrounds. They did not evolve from a single common ancestor. In the diagram below, each circle represents an isolate. Larger circles represents isolates indistinguishable from each other based on cgMLST. 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 pgMLST profiles.

For infections with certain organisms such as *Salmonella* or *Listeria* it is sufficient to compare bacterial genomes**.**

However when investigating CPE spread, comparing the CPE containing isolates, e.g. *Klebsiella pneumoniae*, from different patients, while useful in, may not always be sufficient to understand links between isolates as an incident may be linked by a mobile genetic element (MGE) such as a plasmid that has spread horizontally between different strains and different species.

pOXA-48 and pOXA-181 Plasmid Analysis

Many CPE and ESBL genes are carried and transmitted on mobile genetic elements (MGE's) such as plasmids which can cross the species barrier. Analysis of plasmids from isolates, even those from different species, is important when following the spread of CPE and other antibiotic resistance determinants. *bla*OXA-48 and *bla*OXA-48 like genes, e.g. OXA-181, are the most commonly detected CPE in Ireland, so plasmid content of isolates containing these genes were investigated.

Published pOXA-48 and pOXA-181 sequences were used to create cgMLST schemes for determining the similarity of the IncL/M plasmids and IncX3 plasmids respectively. These schemes are very similar to the one previously designed and used in the reference lab on BioNumerics software.

*bla*OXA-48 is associated with a transposon Tn1999, often present on an IncL (pOXA-48) plasmid. A published pOXA-48 sequence (JN626861.1) was imported and analysed with the Ridom SeqSphere+cgMLST Target definer. The pOXA-48 cgMLST scheme created contained 83 genes. Many of the pOXA-48 isolates contained 79 genes from the subset while others had fewer genes. The results obtained with the new cgMLST scheme were compared to that of the scheme generated using BioNumerics and found to be highly similar.

The same process was performed for blaOXA181 with the IncX3 plasmid KP400525.
A published pOXA-181 sequence (KP400525) was imported and analysed with the Ridom SeqSphere+cgMLST Target definer. The pOXA-181 cgMLST scheme created contained 71

genes. Many of the pOXA-48 isolates contained 69 genes from the subset while others had fewer genes.

Comparisons were then created using both the complete 95.2% cgMLST targets (i.e. 79 genes) for pOXA-48 analysis and 97.2%cgMLST targets pOXA-181 analysis.

Less than complete pOXA48 and pOXA181 plasmids may also be analysed.

- pOXA-48 with ≥ 91.6 % cgMLST targets will be analysed for plasmid group. Those with <91.6 % cgMLST targets will not be analysed.
- pOXA-181 with ≥ 94.4% cgMLST targets will be analysed for plasmid group. Those with <94.4% cgMLST targets will not be analysed.

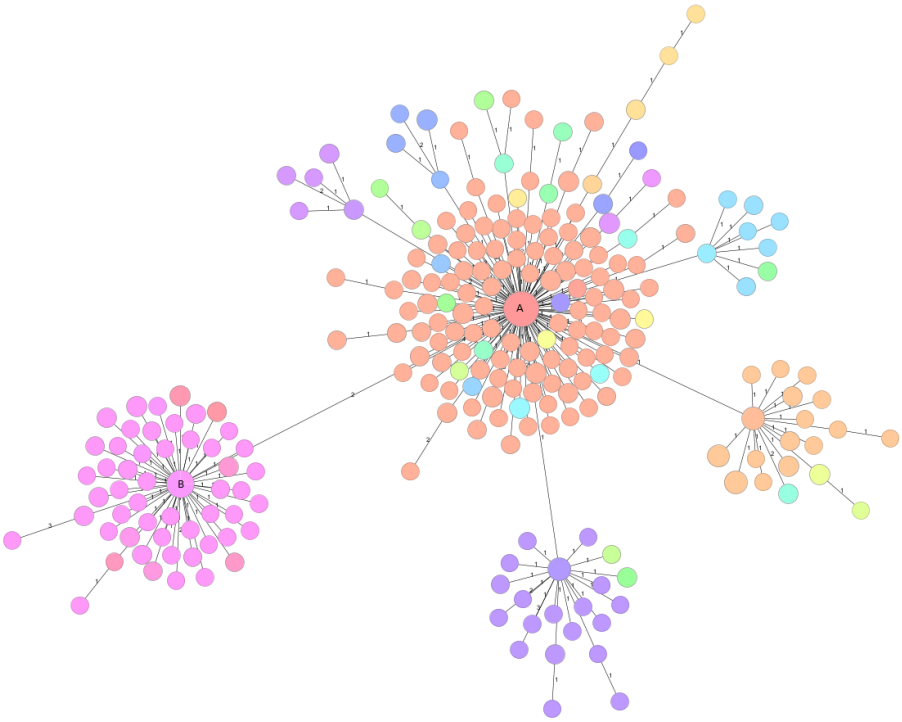

Fig. 3 pOXA48 gene subset of *bla*OXA48 and IncL/M containing isolates coloured by plasmid group.

The two main groups, labelled A and B differ by 2 alleles. Single or groups of isolates up to 4 are given the names A var or B var while clusters of 5 or more are assigned unique group numbers, e.g. A1, B1, etc. Groups off these are called, e.g. A3 var.

Subtyping of plasmids plays a useful epidemiological role in tracking spread of antibiotic resistance determinants. The plasmid group results, e.g. A, A3, A var, B, B2, etc., are reported on APEX (and subsequently hard copy report) and line listings, annual reports and laboratory specific update reports.

Unfortunately, plasmid typing is not available for other plasmid types due to limitations of short read sequencing.

Reports:

Reports are sent to the consultant microbiologist or individual on the office list. If the laboratory wants reports to be issued to a different consultant or a non-consultant an email must be sent to the reference laboratory requesting the change. If a patient has moved to a different hospital report(s) may be issued to this hospital on request.

ENDS