NCPERLS ANNUAL REPORT 2018

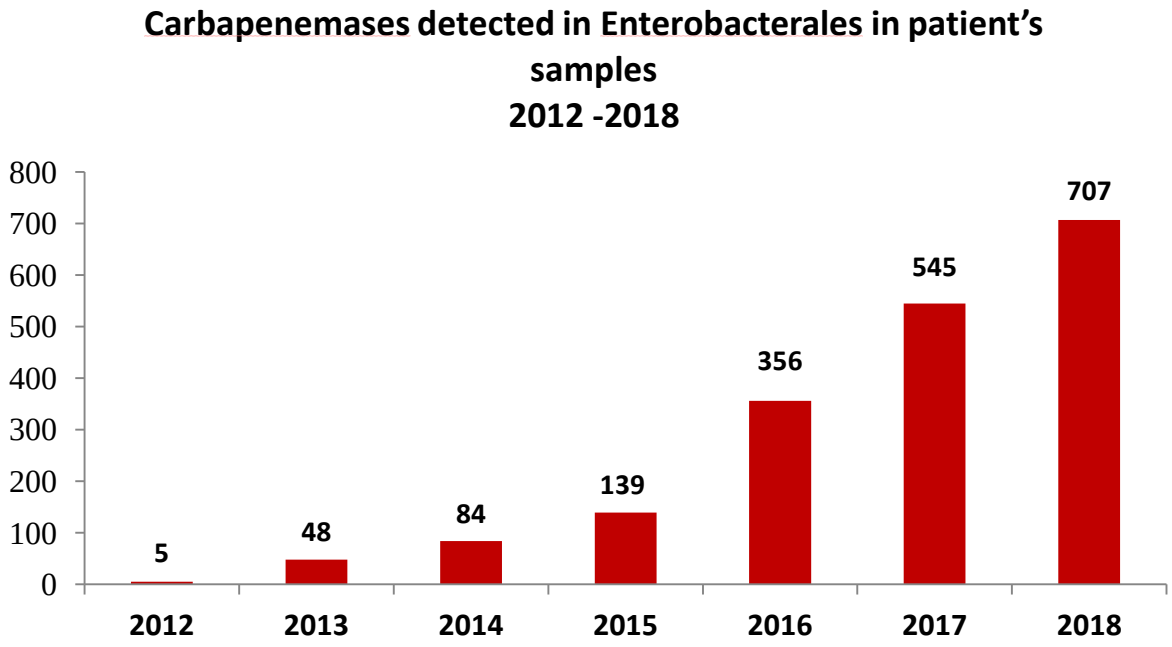
National Carbapenemase Producing Enterobacterales (CPE) Reference Laboratory Service

Summary

This is the sixth annual report since the establishment of the CPE reference laboratory in late 2012. The most important point in this report is that in 2018 537 people in Ireland were newly identified as colonised or infected with CPE. The total number of patient carbapenemase producing Enterobacterales isolates received is considerably higher at 736 because a number of patients are colonised with more than one kind of CPE and repeat samples from patients detected in previous years are not counted.

In 2018 the number of carbapenemase producing Enterobacterales isolates (n=707) submitted increased by 28.5**%** compared to 2017 (n=545). KPC, OXA-48 and NDM remain the most common carbapenemase genes detected in Ireland. CPE was associated mainly with *Klebsiella species* (n=240) and OXA-48 (n=538) was the most common CPE enzyme detected in 2018. There was an increase in the number of OXA-48 producing Enterobacterales in 2018 compared with 2017 (2017 n=417, 2018 n=536). The increasing number of Carbapenemase producing organisms represents a significant threat in particular to the most vulnerable of hospitalised patients although patient to patient spread in other settings also contributes to spread.

Figure 1: Carbapenemases detected in clinical isolates of Enterobacterales in Ireland Sept 2012 to Dec 2018.



1. Establishment and Funding of the Service

The National Carbapenemase Producing Enterobacterales (CPE) Reference Laboratory Service (NCPERLS) was established in October 2012 by the Health Service Executive. In 2018, the HSE also allocated 2 extra posts to support the additional work, with a specialist scientist and another senior medical scientist. These posts will be filled in 2019.

This represents the sixth annual report of the NCPERL service. This report provides some background on the problem of CPE and briefly summarises the output of the service in 2018. Many of the methods used in delivery of the NCPERL service are included on the scope of accreditation of the Department of Medical Microbiology at GUH although whole genome sequencing and bioinformatics analysis is not yet accredited.

2. Carbapenemase Producing Enterobacterales (CPE)

The websites of the HSE-Health Protection Surveillance Centre (HPSC) and the Antimicrobial Resistance and infection control (AMRIC) Team includes details on CPE in Ireland and contains useful fact-sheets for patients and members of the public as well as policy and guidance documents related to CPE.

www.hpsc.ie and www.hse.ie/infectioncontrol

The carbapenem antibiotics include: doripenem, ertapenem, imipenem and meropenem. Meropenem is the carbapenem most widely used for treatment of infection in Ireland at present. The carbapenems now play a very important part in the treatment of infection. They are especially important for treatment of infection with the very broad group of bacteria known as Gram-Negative bacilli. Within that broad group there is an order known as the Enterobacterales which includes well know bacteria such as *E. coli*, *Klebsiella pneumoniae* and *Enterobacter* spp. The name Enterobacterales relates to the association of this order of bacteria within the gastrointestinal tract of humans and animals. Everyone has vast numbers of Enterobacterales in their colon. Some Enterobacterales cause gastrointestinal infection (e.g. *Salmonella, Shigella*, Shiga-Toxigenic/Vero-toxigenic *E. coli*) but most Enterobacterales (e.g. *E. coli*, *Klebsiella pneumoniae* and *Enterobacter* spp.) are harmless when confined to the gut and do not cause disease in most people most of the time. However *E. coli* is the most common cause of cystitis and other urinary tract infections even in otherwise healthy people and all the Enterobacterales are important causes of blood stream

infection, pneumonia and other serious infections particularly in vulnerable groups of patients.

Antibiotics play a vital role in prevention and treatment of infection with Enterobacterales. In recent decades many Enterobacterales have become increasingly resistant to antibiotics. *E. coli* is a useful example on which to base a very general overview of a complex process. Fifty years ago most *E. coli* were very sensitive to ampicillin. Now half or more than half of all *E. coli* causing infection in people in Ireland are resistant to ampicillin. As ampicillin resistance became more common alternative newer agents were needed to treat infection. These included penicillin based combinations (for example amoxicillin-clavulanic acid, piperacillin-tazobactam) and cephalosporins (for example cefotaxime, ceftriaxone). In the last 20 years resistance to these agents have also become increasingly common. An important example of this increasing resistance is the Extended Spectrum Beta-Lactamase producers a.k.a. ESBL's which are generally resistant to cephalosporins such as cefotaxime and ceftriaxone. ESBL's are now very widely disseminated in Ireland, as elsewhere in the world, in hospitals, in nursing homes and in the community and have also been found in food and water.

Recent data from the EARS-net surveillance scheme collated by HPSC indicates that the percentage of those patients with *E. coli* bloodstream infection in which the organism was an ESBL *E. coli* remains steady at 11.0% (2017) to 11.6% (2018) and the percentage that were caused by multidrug resistant (MDR) *E. coli* (displaying resistance to three or more antimicrobial classes) is the highest to date (5.0 in 2014 to 6.2 in 2018). The figure for Carbapenemase Producing *E. coli* was stable at 0.1% (0.2% in 2017). All CPE *E.coli* BSI isolates (n=3) were OXA-48. The percentage of those patients with *Klebsiella pneumoniae* bloodstream infections in which the organism was MDR was up from a low of 5.8 in% in 2016 to its highest level to date, 8.3% in 2018. There were 4 carbapenem resistant MDRKP BSI reported which is the same as in 2017. It is important to note that in 2006 only 2.5% of *E. coli* BSI were ESBL producers. The proportion has quadrupled since that time. The absolute number of people with ESBL *E. coli* has increased to a greater degree because of the overall increase in the number of people developing *E. coli* BSI. While the proportion of CPE E. coli BSI remains low the experience of ESBL illustrates the potential for rapid increase in the absence of effective controls. It is also important to note that blood stream isolates for Enterobacterales are the tip of an iceberg in terms of assessing dissemination of antimicrobial resistant Enterobacterales. For every case of blood stream infection detected there are many cases of individuals that have less serious infections or that are colonised but not infected.

http://www.hpsc.ie/a-z/microbiologyantimicrobialresistance/europeanantimicrobialresistancesurveillancesystemearss/ears-netdataandreports/

As recently as a few years ago the carbapenem antibiotics such as meropenem, represented antibiotics "to depend on" for treatment of life-threatening infection with *E. coli* or indeed a *Klebsiella pneumoniae.* One could expect meropenem to work even when the bacteria were resistant to almost everything else. That situation has changed and carbapenem-resistant Enterobacterales are increasingly common. Carbapenem resistant bacteria (CRE) can be considered in two groups. The group that causes most concern from a public health perspective are the Carbapenemase producers (CPE's) however the "other" CRE also represent a problem for treating patients with infection.

Carbapenemase Producing Enterobacterales (CPE's)

Carbapenemase producers (a.k.a. CPE's) produce enzymes that can inactivate the carbapenems antibiotics. These bacteria are also generally resistant to many penicillins and cephalosporins. The genes for these enzymes are usually on mobile genetic elements that can transfer easily from one bacteria to another. It is in the nature of Enterobacterales that they spread easily from person to person by the faecal-oral route directly (hand to hand) and indirectly (in water and food). Enterobacterales can also survive for long periods in healthcare environments notably in drains from sinks, showers and sluices where they can establish growth as biofilm. These locations can act as reservoirs from which Enterobacterales, including CPE can spread to patients. In addition to resistance to penicillins, cephalosporins and carbapenems, CPE's are very often resistant to many other families of antibiotics. In some cases there are very few antibiotics available.

The Non-Carbapenemase Producing but Carbapenem Resistant Enterobacterales

In some cases Enterobacterales are resistant to carbapenems although they do not produce one of the known carbapenemase enzymes. In most cases these bacteria are resistant due to a small number of changes. Usually there are one or more changes that prevent the carbapenem antibiotic from entering the bacteria efficiently. This, together with other enzymes produced by the bacteria, which are not very efficient against carbapenems, but are sufficient to break down the small amount of carbapenem that can penetrate into the cell in the context of other changes.

Treating Infection with Carbapenem-Resistant Enterobacterales

Whether due to true CPE or a mixture of other changes the range of antibiotics available for treating infections with Enterobacterales that are resistant to carbapenems is limited. The situation is made worse because few new families of antibiotics have become available for clinical use in the last 30 years. All Enterobacterales that are resistant to carbapenems, therefore represent a threat to the most vulnerable patients within the health care system but the threat is generally greatest with CPE because of their propensity for spread. Guidance on the treatment of CPE infection was issued by the CPE Expert Group in early 2019.

One option for treatment of CPE infection is the combination ceftazidime-avibactam. This combination is active against OXA-48 like and KPC variants of CPE though not against metallo-carbapenemases such as NDM. While this combination is included in the options for treatment in the guideline referred to above it is worth noting that resistance to this combination has already been reported and needs to be monitored.

https://ecdc.europa.eu/en/publications-data/rapid-risk-assessment-emergence-resistance-ceftazidime-avibactam-carbapenem

Between July 2018 and December 2018 the ceftazidime-avibactam M.I.C. of all CPE isolates submitted to the reference laboratory service was determined by reference broth microdilution. The only isolates testing resistant are class B (metallo beta-lactamases) against which the combination is expected to be inactive.

When CPE cause serious infection they are an immediate risk to the life of the patient concerned. Even if the CPE are just resident in the gut it is a long-term risk to that patient, because it may subsequently cause infection. Colonisation with CPE is also a risk to all other patients because the bacteria may spread to other, even more vulnerable patients particularly in the hospital, clinic or nursing home. It is important to stress that once a patient has acquired a CPE in their gut there is no accepted process that is likely to be successful at eradication although recent guidance from the CPE Expert Group specifies conditions under which the designation as CPE Colonised, for infection prevention and control purposes, can be removed from a patients' record.

Carbapenem-Resistance in Other Families of Bacteria

The situation is made more complicated because some members of certain other orders of Gram-negative bacteria may be naturally occurring carbapenemase enzymes. Examples include *Acinetobacter species* and *Stenotrophomonas maltophilia*. Other environmental

bacteria are naturally susceptible to carbapenems but very readily become resistant for example *Pseudomonas aeruginosa*. In some cases these bacteria (especially *Acinetobacter species*) may spread rapidly in healthcare environments and may acquire additional carbapenemase enzymes. It is a concern that a number of *Acinetobacters spp*. with transferrable high level carbapenem resistance have been detected in Ireland recently. This organism could represent a significant threat if it is established in a hospital and disseminates widely. In 2018 there were a small number of cases of NDM *Acinetobacter spp.* This finding was highlighted to Hospital Group CEO's, Infection Prevention and Control Practitioners, Consultant Microbiologists, Consultant Infectious Disease Physicians, Public Health Departments, Chief Medical Scientists in Microbiology and Surveillance Scientists in September. The following was recommended:

1. Perform meropenem susceptibility testing by a validated method on all clinically significant *Acinetobacter spp*. from diagnostic samples.

2. Perform meropenem susceptibility testing by a validated method on a proportion of *Acinetobacter spp.* cultured from screening samples on chromogenic agar. Depending on capacity and local factors a laboratory could consider testing such isolates from high risk areas (for example ICU) or a random sample of isolates (for example every fifth such isolate).

3. For isolates of *Acinetobacter spp*. that test resistant to meropenem consider in-house testing for NDM (lateral flow and molecular methods are likely to be effective*).

4. Consider submitting all NDM positive *Acinetobacter spp.* to national reference laboratory for further characterisation.

5. Bank all meropenem-resistant non-NDM *Acinetobacter spp*. so that they are available for retrospective analysis if required.

***Note re commercial lateral flow and commercial methods.** Most commercial methods detect the main 5 CPE i.e. OXA-48, KPC, NDM, VIM & IMP. They generally do not detect OXA-24/40-, OXA-23- and OXA-58-like enzymes which are associated with acquired meropenem resistance in Acinetobacter.

The Emergence of Transferable Colistin Resistance

For some years now one of the treatment options for patients infected with carbapenem-resistant organisms has been colistin. This is an old antibiotic that was not widely used for systemic treatment until very recently because of concerns regarding dosing and toxicity. It has become a critically important therapeutic agent recently because of the progressive loss of other options due to increasing resistance. In 2015 a group of researchers based in China

reported widespread dissemination of Enterobacterales that are resistant to colistin by virtue of a transferrable gene *mcr*-1. This report was rapidly followed by reports of detection of this gene and related genes in many other countries including other EU member states. The first cases of the *mcr*-1 gene have been detected in three human clinical isolates from two patients in Ireland during 2017. There were no *mcr*-1 isolates detected in 2018.

Changing Technology

Methods for classification and sub classification (typing) of bacteria are undergoing a very rapid transformation. In particular determining and comparing the entire DNA sequence of bacteria (whole genome sequencing, WGS) for the purpose of tracking routes of spread of infection is increasingly important. Following the acquisition of an instrument for whole genome sequencing in late 2017 the reference laboratory service in 2018 is increasingly based on whole genome sequencing and bio-informatic analysis. As of July 2018 all isolates were analysed by WGS instead of real time PCR. This increased the turnaround time for reports but allowed a much more detailed analysis of the isolate to be achieved, which aided greatly in following outbreaks and help trace pathways of spread.

The roles of the National CPE Reference Laboratory Service are

1. To support clinical laboratories by differentiating between CPE's and carbapenem resistance due to other reasons
2. To provide extended antibiotic susceptibility testing on CPE's when requested
3. To help trace pathways of spread of CPE's by assessing the degree of similarity between CPE's and between CPE plasmids from different patients and from different hospitals
4. To provide support in investigation of suspected outbreaks of CPE infection
5. To provide national data to inform public health policy on the scope of the problem and the effectiveness of responses

This annual report contributes to achieve the objective of informing clinicians and policy makers. For information on submission of isolates please see users guide (Appendix 3).

Summary of Data for 2018

3.1 Methodology

For all isolates submitted, species identification was confirmed using MALDI-TOF mass spectrometry. Colistin MIC was determined on all Carbapenemase producing isolates using the TREK Sensititre (semi-automated microbroth dilution), with ceftazidime-avibactam

tested from July onwards. From January to June, routine phenotypic detection and characterisation of Carbapenem resistant Enterobacterales was determined using a commercial kit – ROSCO KPC/MBL and OXA-48 Carbapenemase Confirm Kit, and were also analysed by molecular methods for all the more common genes associated with CPE (OXA-48, KPC, NDM, VIM and IMP) and a number of uncommon genes when required (IMI, GES, OXA-23, OXA-24/40, OXA-51 and OXA-58). From July onwards all isolates were analysed by whole genome sequencing. Also, isolates from laboratories without on-site rapid CPE detection assays were tested using the NG Biotech Immunochromatographic assay for the 5 main CPE (OXA-48, KPC, NDM, VIM and IMP), in order for those laboratories to get a rapid preliminary result while awaiting WGS reports.

In addition to examination for CPE genes, as appropriate, isolates are examined by molecular methods for certain non-CPE genes that may contribute to make the bacteria resistant to carbapenems. The detection of these genes can help to provide an explanation for non-carbapenemase mediated carbapenem resistance (Table 6 & 7).

All submitted isolates are stored frozen at -80°C for a minimum of 3 years to permit re-evaluation and to enable additional studies in the event that new concerns arise, to support new method validation and to allow potential for additional analysis should new methods become available. Reports from the NCPERLS provide the referring laboratory with a detailed report of all analyses performed for their records and with an interpretive comment where appropriate. Where the laboratory is alerted to particular urgency in a specific case an effort is made to expedite the preliminary report.

Printed reports are issued by mail to the named responsible person in referring laboratory. The NCPERLs does not notify the isolates as the referring laboratory undertakes notification. When there is evidence of cross transmission of bacteria within a hospital or between hospitals the relevant contact people in the hospital(s) are informed.

3.2 CPE in Ireland in 2018

From January to December 2018 1565 isolates were submitted to the NCPERLS by clinical laboratories throughout Ireland. This represents an increase of 35% on the number submitted in 2017. Managing this increase was possible because of the commitment of the medical scientists involved, the support of other staff in the Department of Medical Microbiology at GUH and the continuing support of the Saolta Group. Where multiple copies of an isolate were received from a given patient only the first isolate is included in the data.

Of the 748 carbapenemase producers 707 were Enterobacterales isolates, 32 *Acinetobacter species*, 7 *Pseudomonas species* and 2 non-culturable samples. The majority of Enterobacterales are accounted for by *E.coli* (n=194) and *Klebsiella species* [*K.oxytoca* (n=84) & *K.pneumoniae/variicola* (n=156)]. Figure 2 shows the extended breakdown of all carbapenemases producing organisms. A number of patients were colonised with more than one kind of CPE or duplicates of isolates or isolates from patients detected in previous years were received by the NCPERLs i.e. 707 isolates were from 537 newly identified patients. An increase in environmental samples with carbapenems detected was noted in 2018, (Table 2) with a total of 75 isolates referred, of which 65 had carbapenems detected. Of these, 8 were intrinsic resistance genes in *Acinetobacter species* (OXA-51 and OXA-23) and 2 VIM in Pseudomonas aeruginosa. Carbapenemase producing Enterobacterales environmental isolates accounted for 55 of the total detected.

It is important that laboratories use methods of testing that allow them to detect those isolates with reduced susceptibility (increased minimum inhibitory concentration) even though they may not cross the threshold for clinical resistance. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf. Any isolates that test negative in the rapid CPE assays, which show reduced inhibition to carbapenems should be referred to the NCPERLs for further analysis.

Isolates with carbapenemases detected were from blood (*n* = 15), rectal swab/faeces (*n* = 633), (*n* = 50), respiratory samples (*n* = 13), other sites (*n* = 32), not stated (5) environmental samples (*n* = 65) - Table 3.

In 2018, Carbapenemase genes were associated mainly with *Klebsiella species*, with OXA-48 dominating as the most common enzyme detected followed by KPC, NDM and VIM (Table 4 patient isolates). The increase in OXA-48 seen in 2017 (n=417) has continued in 2018 (n=538). In 2018 KPC (n=94) numbers have continued to increase also following a reduction in 2016 (Figure 3). The increase in OXA-48 Enterobacterales was largely due to an ongoing outbreak in all hospital groups with the exception of the Children's and University of Limerick hospital groups - as illustrated in Table 5. KPC remains predominately associated with the University of Limerick Hospital Group.

Co-carriage of more than one CPE enzyme has been seen in a number of isolates:

- One rectal swab, *Escherichia coli* with NDM and IMP
- Two rectal swabs, *Citrobacter species* with NDM and OXA-48
- One rectal swab, *Escherichia coli* with NDM and OXA-48
- One rectal swab, *Klebsiella oxytoca* with NDM and OXA-48
- One rectal swab, *Escherichia coli* with KPC and IMP
- One rectal swab, *Acinetobacter baumannii* complex NDM, OXA-58 and OXA-51
- One pressure sore swab, *Acinetobacter baumannii* complex NDM and OXA-51
- Two rectal swab, *Acinetobacter baumannii* complex OXA-23 and OXA-51

A number of patients have had multiple isolates detected with the same type of CPE:

- 93 patients with 2-4 different isolates with OXA-48 detected
- 10 patients with 2-3 different isolates with KPC detected
- 1 patient with 2 different isolates with IMP detected

- 3 patient with 2 different isolates with NDM detected
- 1 patient with 2 different isolates with VIM detected
- 1 patient with 2 different isolates with OXA-23 detected

This reflects the high mobility of the genetic elements that code for many CPE genes. Patients have had multiple CPE detected in different species:

- OXA-48 in two different *Enterobacter cloacae* complex strains (one with CTX-M group 1 and other with CTX-M group 9)
- OXA-48 in *K.pneumoniae* and NDM in *Citrobacter species*
- OXA-48 *Citrobacter species* and VIM *Pseudomonas alcaligenes*
- OXA-48 *K.variicola* and *K.oxytoca* and IMP *Pseudomonas aeruginosa*
- OXA-48 in *K.oxytoca* and *Pluralibacter gergoviae* in two healthcare institutions, and NDM in *Acinetobacter baumannii* complex
- NDM *E.coli* in one strain and OXA-48 in another strain of *E.coli*
- NDM *E.coli* and OXA-48 *K.pneumoniae*
- NDM & OXA-48 in both *Citrobacter species* and *K.oxytoca*
- KPC *Citrobacter species* and *K.pneumoniae* and OXA-48 *K.oxytoca*

Referral of CPE isolates from the same patient from different healthcare institutions occurred in 24 cases.

From January to June 2018, when in-house real time PCR was the method of analysis, where appropriate, isolates were examined by molecular methods for non-CPE genes, including CTX-M and plasmid-mediated *ampC* genes. Findings are displayed in Table 6. Of the 232 isolates, the additional resistance genes detected were mainly *bla*CTX-M group 1 (n=156), followed by *bla*CTX-M group 9 (n=37). From July 2018 WGS was introduced as the method of analysis, with findings displayed in Table 7. CTX-M ESBL resistance was the most common (n=119) and co-production of ESBL and AmpC was common also (n=88).

During the months January to June 2018, the NCPERL tested every isolate received for *mcr*-1 gene by real-time PCR. With the adoption of whole genome sequencing in July 2018 all isolates referred to the reference laboratory were assessed for *mcr* genes. In 2018 *mcr* genes were not detected.

Progress in 2019

For 2019 the reference laboratory service aims to continue to provide a timely and quality service to its users, to optimise use of resources and to enhance capacity in relation to Whole Genome Sequencing.

Table 1: Number of Enterobacterales submitted and Molecular findings (rtPCR or WGS) in 2018

Species	Number Submitted	Number Confirmed CPE
E.coli	379	194
Klebsiella spp.	425	240
Enterobacter spp.	398	167
Citrobacter spp	86	75
Other Enterobacterales	77	31
Total	**1365**	**707**

Note: *Acinetobacter spp* (submitted 47 isolates, 32 with carbapenemase detected): *Pseudomonas spp* (submitted 32 isolates, 7 with carbapenemase detected): Other bacteria (Submitted 5 isolates, none with carbapenemase detected) and isolates that did not grow on sub-culture (submitted 4, 2 with carbapenemase detected)

Figure 2: Number of isolates confirmed with carbapenemases in 2018

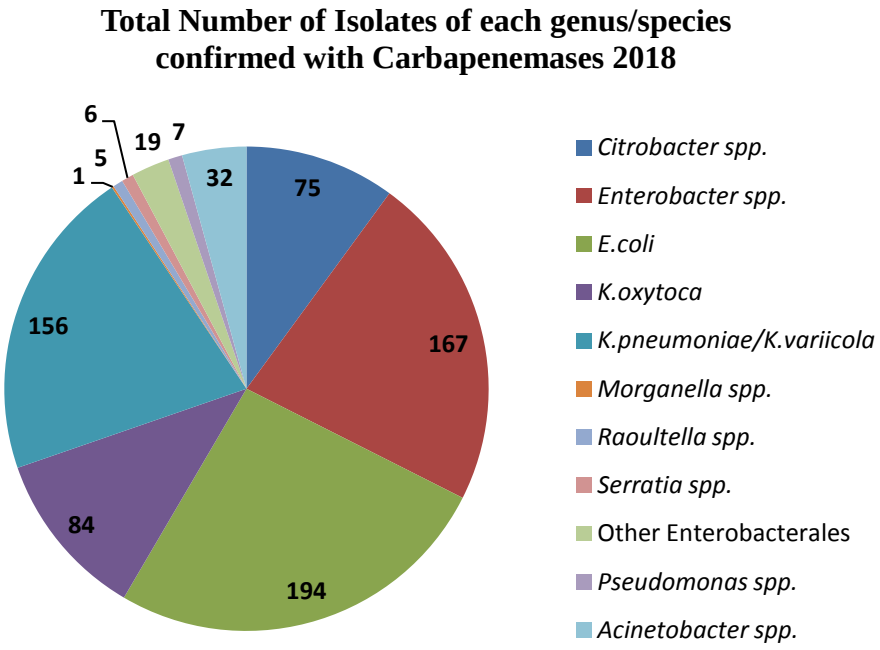


Table 2: Isolates from the healthcare environmental with carbapenemases detected in 2018

Species	KPC	OXA-48	NDM	VIM	IMP	IMI	OXA-23	OXA-51
Acinetobacter spp.	0	0	0	0	0	0	2	8
Citrobacter spp.	1	4	0	1	0	0	0	0
Enterobacter spp.	0	16	1	9	2	0	0	0
Klebsiella spp.	0	5	0	0	2	0	0	0
***Other Enterobacterales**	0	1	0	6	0	0	0	0
Pseudomonas aeruginosa	0	0	0	2	0	0	0	0
Raoultella spp.	3	2	0	0	0	0	0	0
Total (n=65)	**4**	**28**	**1**	**18**	**4**	**0**	**2**	**8**

*** Other Enterobacterales: *Leclercia adecarboxylata* (n=6)**

Table 3: Carbapenemase Producing Isolates by antatomica site of sample

CPE Enzyme	Screening[1]	Urine	Respiratory	Blood	Other site	Not stated	Environmental
OXA-48	445	42	9	12	25	5	28
KPC	87	5	0	0	2	0	4
NDM	37	1	0	1	3	0	1
IMP	14	2	2	0	0	0	4
VIM	22	0	1	2	1	0	18
IMI	5	0	0	0	0	0	0
OXA-23	6	0	0	0	0	0	2
OXA-24	0	0	0	0	0	0	0
OXA-51	14	0	0	0	1	0	8
OXA-58	3	0	1	0	0	0	0
Total (n=813)	**633**	**50**	**13**	**15**	**32**	**5**	**65**

1. Screening samples are rectal swabs or samples of faeces.

Table 4: Type of Carbapenemase detected in patient isolates[#] by genus/species

Species	KPC	OXA-48	NDM	VIM	IMP	IMI	OXA-23	OXA-24	OXA-58
E.coli	12	159	19	2	2	0	0	0	0
Klebsiella spp	37	190	7	4	2	0	0	0	0
Enterobacter spp	17	122	3	13	7	5	0	0	0
Citrobacter spp	27	40	4	2	2	0	0	0	0
Other Enterobacterales	1	25	1	0	4	0	0	0	0
Pseudomonas spp	0	0	1	5	1	0	0	0	0
Acinetobacter spp*	0	0	7	0	0	0	6	0	4
Unknown	0	2	0	0	0	0	0	0	0
Total (n=733)	**94**	**538**	**42**	**26**	**18**	**5**	**6**	**0**	**4**

[#]Excluding environmental isolates

*Excluding OXA-51 intrinsic resistance gene (n=15)

Table 5: Total number of newly detected patients with Enterobacterales CPE in Ireland Hospital Group 2018

Hospital group	KPC	OXA-48	NDM	VIM	IMP	IMI
Children's Hospital Group	0	3	0	2	0	0
Dublin Midlands	2	106	9	0	0	0
Ireland East	9	46	3	0	0	0
RCSI	3	57	1	1	0	1
Saolta	3	78	3	17	0	1
South/South West	8	77	3	0	0	0
University Limerick	50	6	1	0	0	1
*Other	5	22	5	0	12	2
Total (n=537)	80	395	25	20	12	5

***Other:** Non-HSE Hospitals, Nursing Homes/LTCF or GP samples.

Note: This data is based on bacterial cultures submitted to the National CPE reference laboratory service based at Galway University Hospital. Patients are counted once only in the hospital/hospital group from which their first CPE isolate was submitted to the reference laboratory. It should not be assumed that the location of the patient at the time of first detection represents the hospital/hospital group in which colonisation/infection was acquired. All Non-Enterobacterales and environmental isolates are excluded from this data. Hospital groups differ substantially in the terms of bed numbers and scope of services provided. Furthermore differences in number of isolates are likely to be related in a substantial measure to difference in screening practices.

Table 6: Additional Resistance Genes Findings (January-June 2018)

Species	CTX-M Grp 1	CTX-M Grp 2	CTX-M Grp 9	Pm-ampC[1]	CTX-M + Pm-ampC
E.coli **(n = 92)**	52	0	14	15	11
Klebsiella spp **(n = 68)**	52	0	3	13	0
Enterobacter spp **(n = 63)**	49	0	14	NT	NT
Other (n=9)	3	0	6	NT	NT
Total (n=232)	**156**	**0**	**37**	**28**	**11**

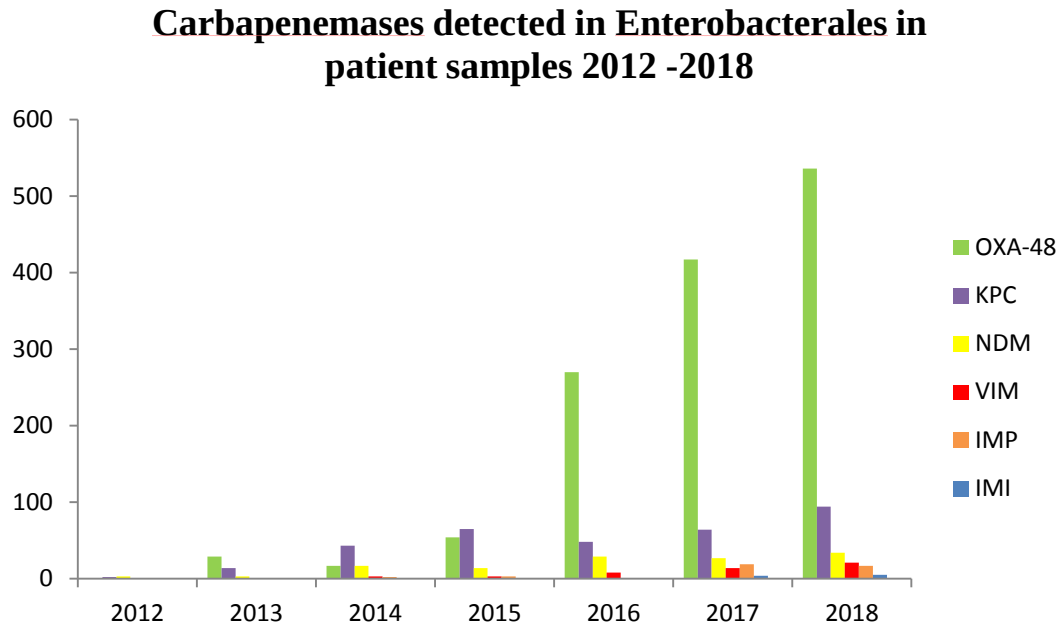
1. Plasmid mediated ampC.

Table 7: Additional Resistance Genes Findings (July-December 2018)

Species	CTX-M ESBL only*	SHV ESBL	TEM ESBL	AmpC only	ESBL + AmpC
E.coli **(n=63)**	52	0	0	6	5
Klebsiella spp **(n=79)**	60	9	1	8	1
Enterobacter spp **(n =85)**	0	0	0	7	78
Other (n=33)	7	5	0	17	4
Total (n=260)	**119**	**14**	**1**	**38**	**88**

*3 with SHV ESBL also counted here

Figure 3: Common Carbapenemases detected in Enterobacterales 2012 – 2018



4 Whole Genome Sequencing Data

Whole Genome Sequencing (WGS), introduced in July 2018, is performed on illumina MiSeq platform, using Nextera XT kit. Data analysis of generated WGS data is carried out using commercial software BioNumerics Version 7.6.3, supplemented by other external software as required. The Reference Service aims to report on Sequence Type (ST), resistance genes and plasmids. In relation to resistance genes, at present we are primarily reporting beta-lactamases, namely carbapenemases, ESBLs and ampCs. For plasmids, our focus has been on plasmids carrying the OXA-48 carbapenemase gene and its variants – IncL/M(pOXA-48) and IncX3.

The figures detailed below detail the relationships between isolates of same the species, illustrated using Minimum Spanning Trees (MST) generated using BioNumerics software.

Figure 4: Carbapenemase Producing *Klebsiella pneumoniae* 2018 cgMLST MST – by hospital

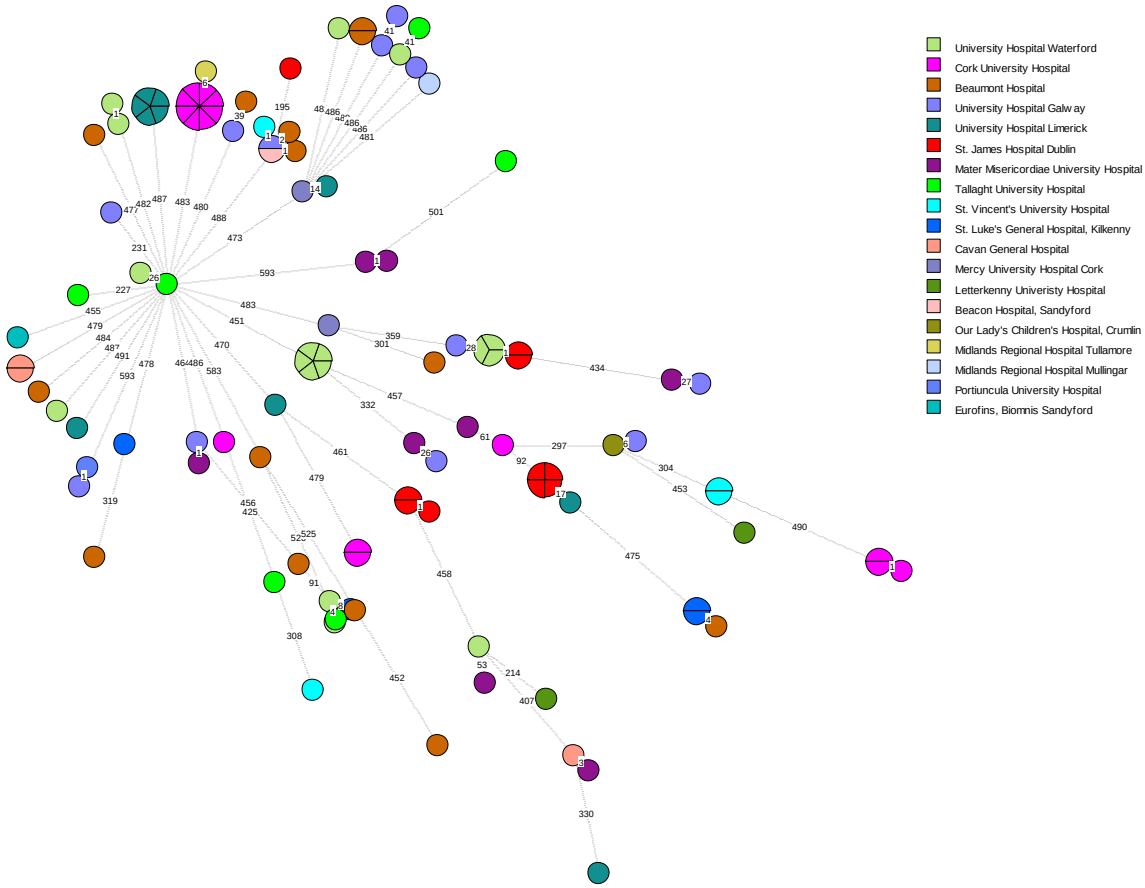


Fig. 4 Illustrates the relationship between carbapenemase producing *K. pneumoniae* isolates received in 2018, with each circle representing a single isolate and each colour representing one hospital. The relationship tree is based on the core genome of *K. pneumoniae* which consists of 634 core loci. The number listed on each branch represents the number of loci differences between each isolate. The patterns shows great overall diversity but note multiple instances of clusters of indistinguishable or very closely related isolates from individual hospitals consistent with person to person transmission (direct or indirect) within that hospital or acquisition by multiple patients from a common source (for example an environmental reservoir).

Figure 4.1: Carbapenemase Producing *Klebsiella pneumoniae* 2018 cgMLST MST – by carbapenemase gene

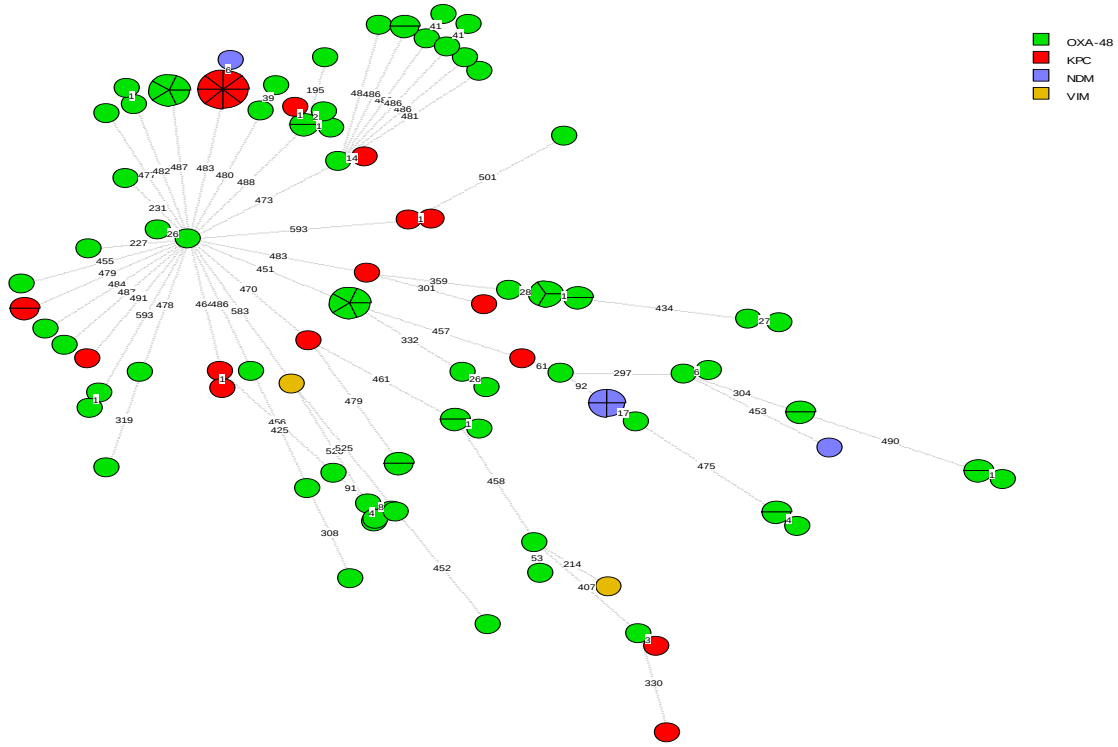


Fig. 4.1 is the same relationship tree as illustrated in Fig. 4, coloured by carbapenemase gene. OXA-48, coloured in green, represents the majority of carbapenemase producing *K. pneumoniae* received in 2018. There are a limited number of carbapenemase genes that are widely disseminated in very diverse strains.

Figure 4.2: OXA-48 *Klebsiella pneumoniae* - 2018 cgMLST MST (by hospital)

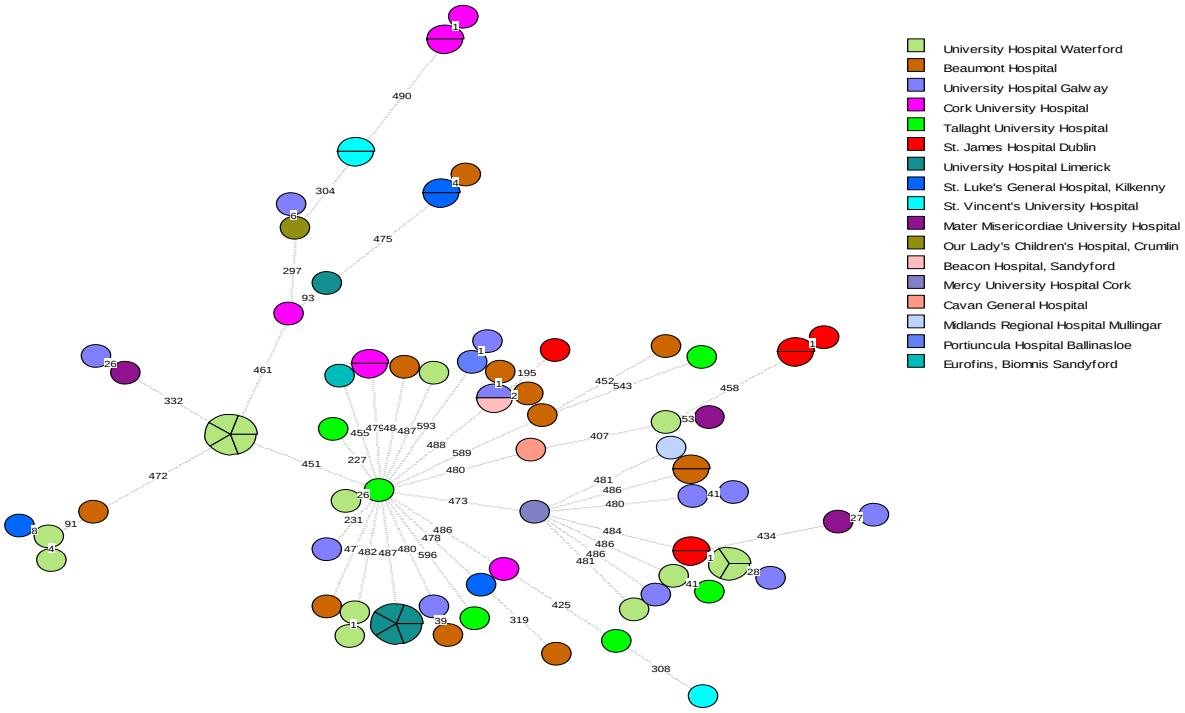


Fig. 4.2 is a detailed look at OXA-48 *K.pneumoniae*, coloured by hospital. The patterns shows great overall diversity but note multiple instances of clusters of indistinguishable or very closely related isolates from individual hospitals consistent with person to person transmission (direct or indirect) within that hospital or acquisition by multiple patients from a common source (for example an environmental reservoir). Note that the IncL/M(pOXA-48) plasmid is detected in most of the isolates.

Figure 4.3: KPC *Klebsiella pneumoniae* cgMLST MST (by hospital)

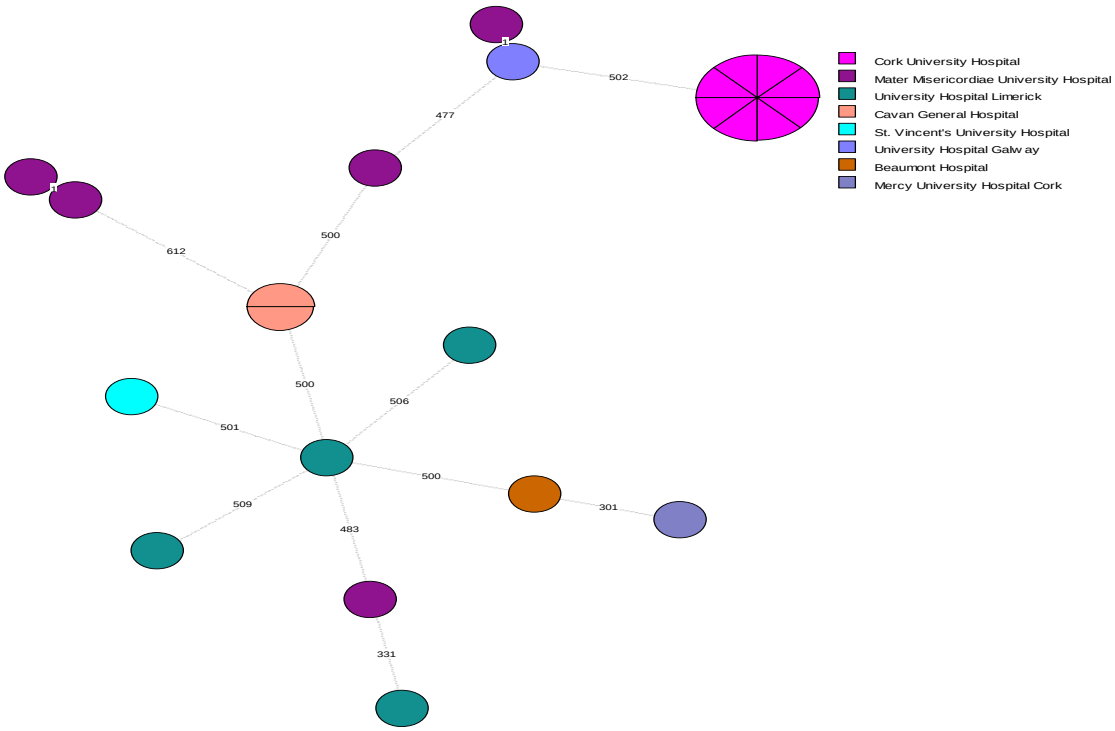


Fig 4.3 is a detailed look at KPC *K. pneumoniae*, coloured by hospital. Although University Hospital Limerick accounts for majority of KPC isolates in 2018, they have mainly been from organisms other than Klebsiella species. There is evidence of a distinct hospital clonal outbreak in Cork University Hospital consistent with person to person transmission (direct or indirect) within that hospital or acquisition by multiple patients from a common source (for example an environmental reservoir). There are also clusters of 2 isolates associated with other hospitals.

Figure 5: Carbapenemase producing *E.coli* 2018 cgMLST MST – by hospital

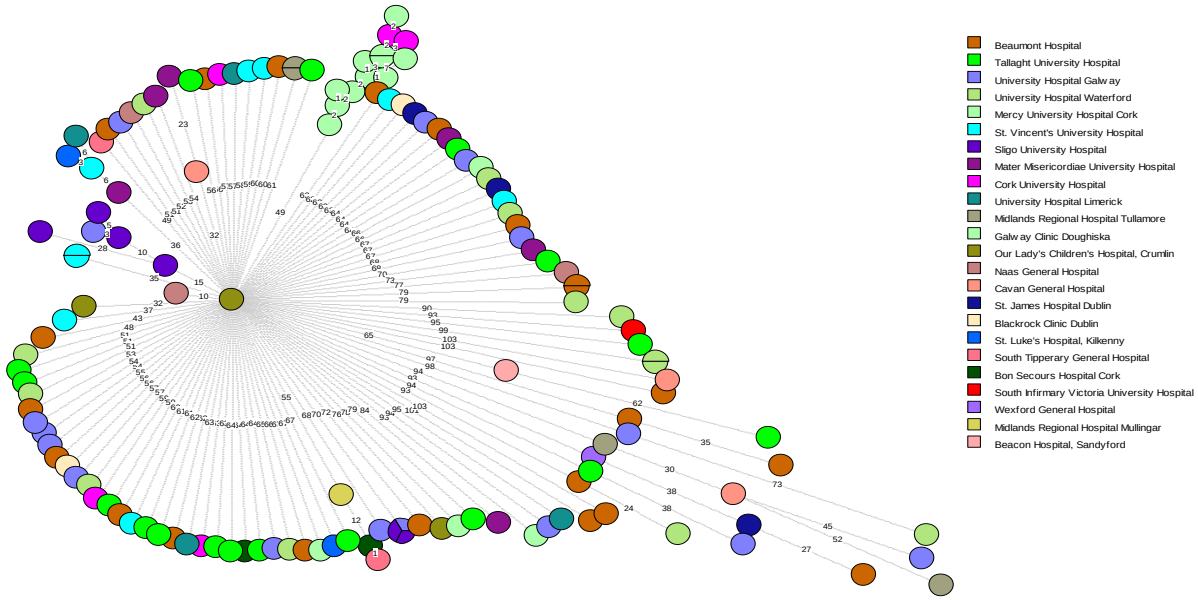


Fig 5 illustrates the relationship between carbapenemase producing *E.coli* isolates received in 2018, with each circle representing a single isolate. The relationship tree is based on the core genome of *E.coli* which consists of 2513 core loci. The number listed on each branch represents the number of loci differences between each isolate. The MST is coloured by hospital. The pattern shows great overall diversity. Note there are clusters of indistinguishable or very closely related isolates associated with some individual hospitals. This is consistent with person to person transmission (direct or indirect) within that hospital or acquisition by multiple patients from a common source (for example an environmental reservoir).

Figure 5.1: **Carbapenemase producing *E.coli* 2018 cgMLST MST – by carbapenemase gene**

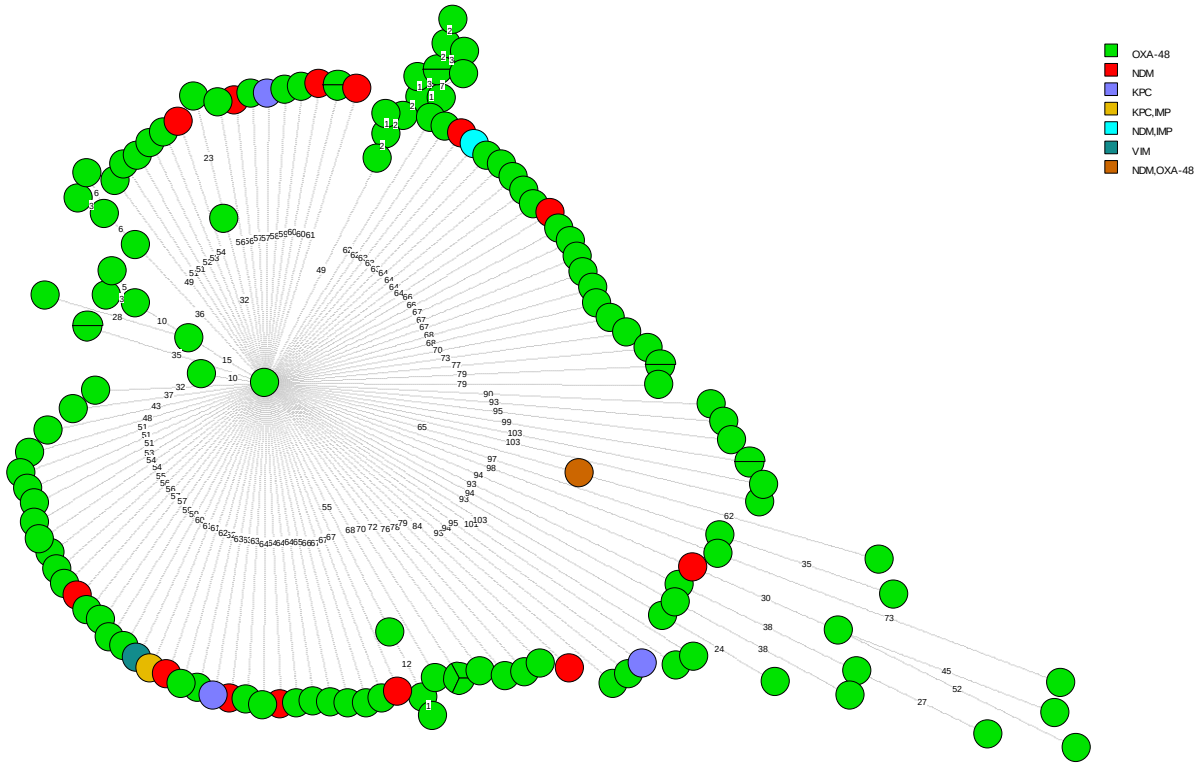


Fig 5.1 is the same relationship tree as illustrated in Fig. 5, coloured by carbapenemase gene. OXA-48, coloured in green, represents the majority of carbapenemase producing *E.coli* received in 2018.

Figure 5.2: ***E. coli*** **producing OXA-48-like carbapenemase[1] cgMLST MST – by carbapenemase gene**

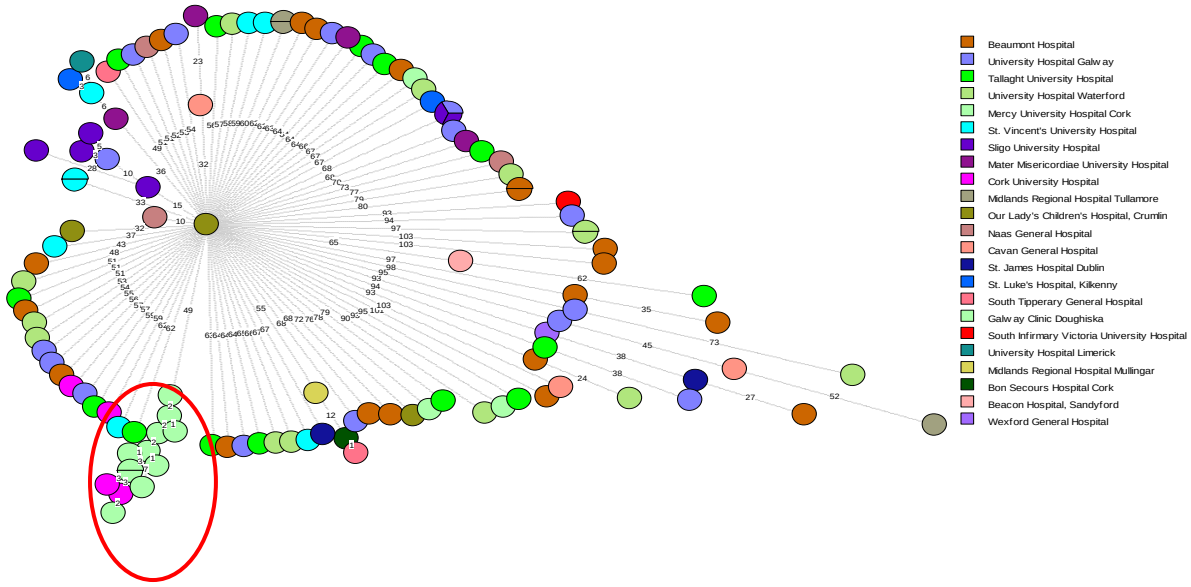


Fig 5.2 is a detailed look at the 2018 OXA-48 *E.coli*, coloured by hospital. There is great diversity of strains. Circled in red, represents an OXA-181 *E.coli* ST-410 outbreak involving 2 hospitals. Most strains of *E. coli* carrying the OXA-48 carbapenemase carry a common plasmid IncL/M(pOXA-48).

1. The term OXA-48- like carbapenemases includes both OXA-48 and the closely related OXA-181 enzymes.

Figure 6: Carbapenemase producing *E. cloacae complex* 2018 wgMLST MST – by hospital

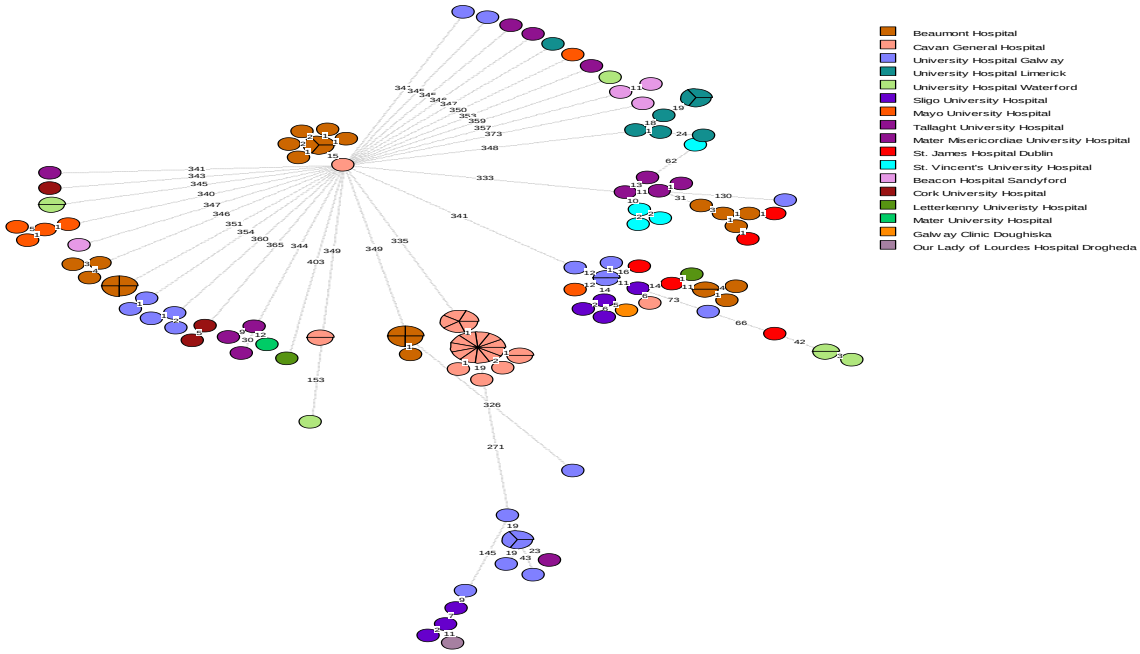


Fig. 6 illustrates the relationship between carbapenemase producing *E.cloacae complex* isolates received in 2018, with each circle representing a single isolate. The relationship tree is based on the whole genome of *E.cloacae complex* which consists of 15,605 loci. The number listed on each branch represents the number of loci differences between each isolate. The MST is coloured by hospital group. Note that there is very significant clustering by hospital. A high proportion of isolates from the hospital environment are *E. cloacae*. Based on the pattern of hospital clustering and the association with the environment on can speculate that the hospital environment may be particularly important as source of acquisition of CPE *E. cloacae*. Given the mobility of the IncL/M plasmid such a reservoir could play an important role in seeding OXA-48 like CPE in other Enterobacterales.

Figure 6.1: Carbapenemase producing *E. cloacae complex* 2018 wgMLST MST – by carbapenemase gene

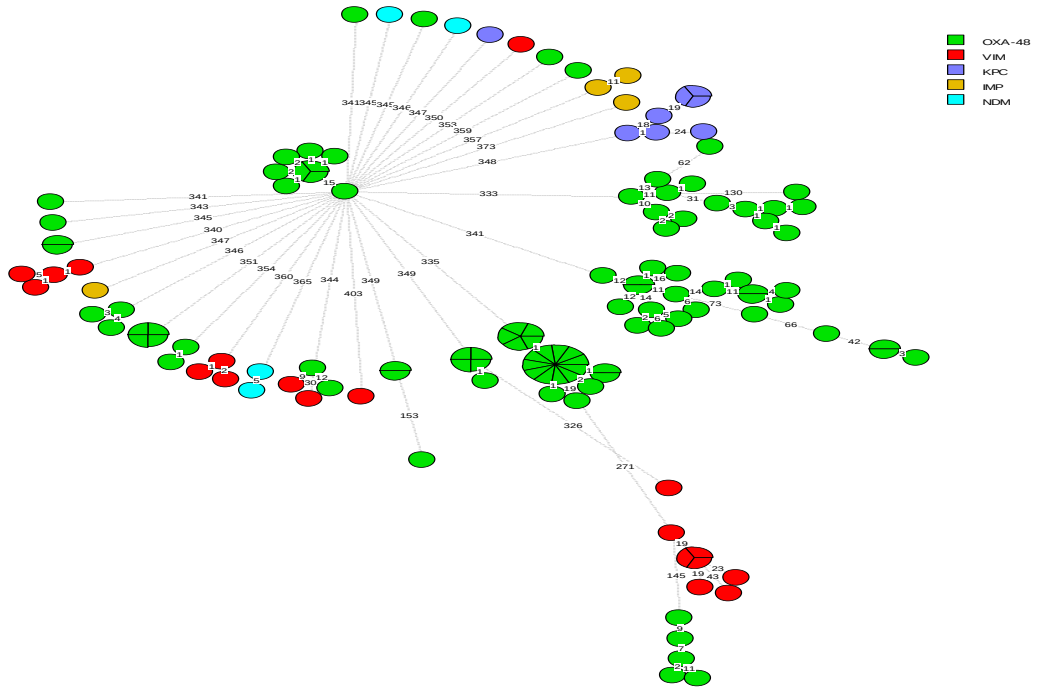


Fig 6.1 is the same relationship tree as illustrated in Fig. 6, coloured by carbapenemase gene. OXA-48, coloured in green, represents the majority of carbapenemase producing *E.cloacae complex* received in 2018.

Figure 6.2: Carbapenemase producing *E. cloacae complex* 2018 wgMLST MST – by Sequence Type

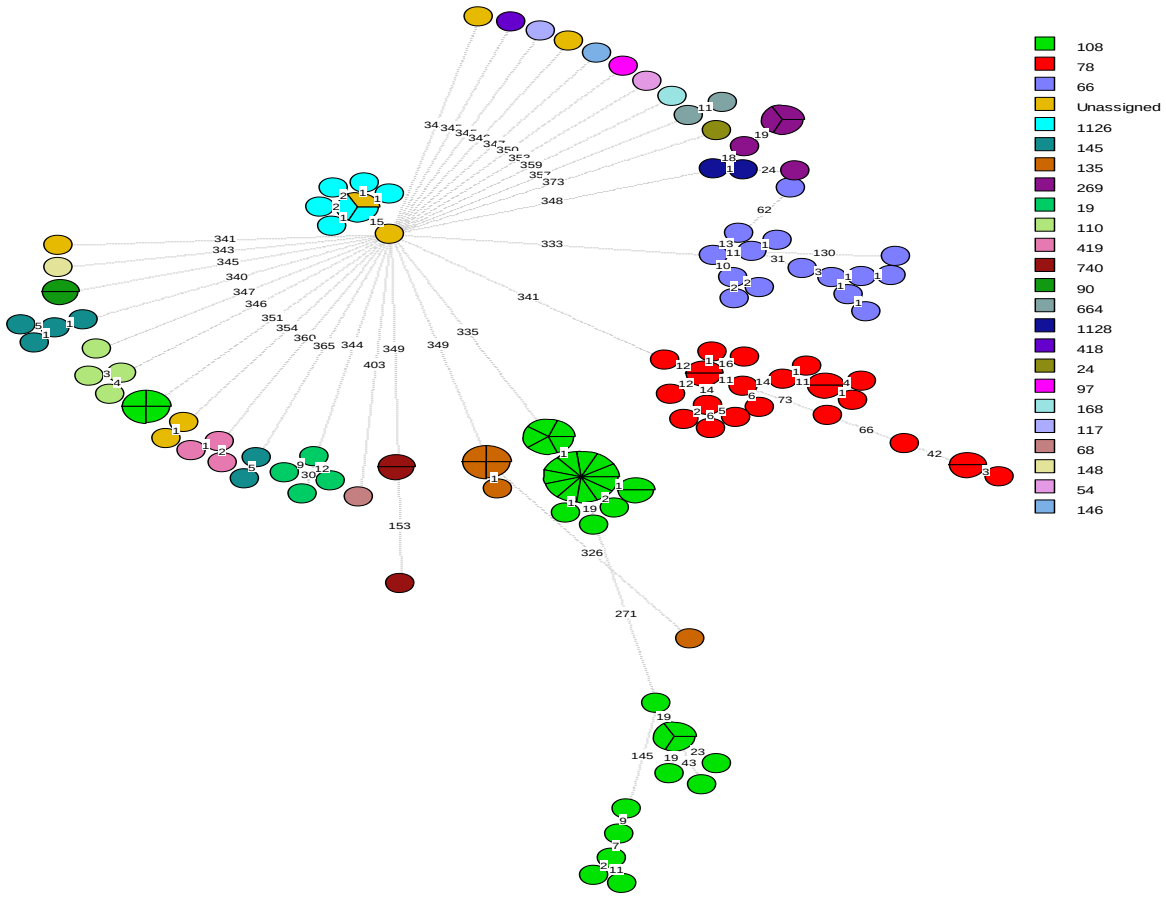


Fig 6.2 is the same relationship tree as illustrated in Fig. 6 and Fig.6.1, coloured by sequence type. *E.cloacae complex* ST-108, -78, -117 and -1216 have been successful sequence type circulating in 2018 responsible for a number of hospital OXA-48 outbreaks.

Figure 7: pOXA-48 MST – Enterobacterales – by hospital

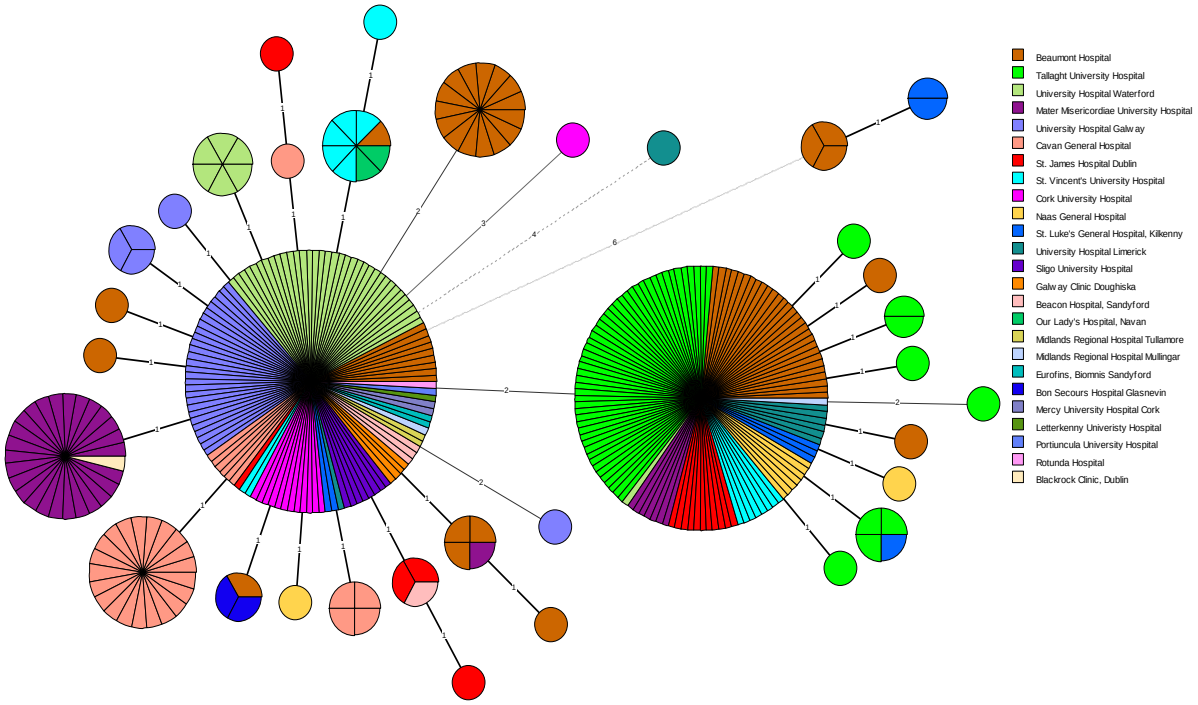
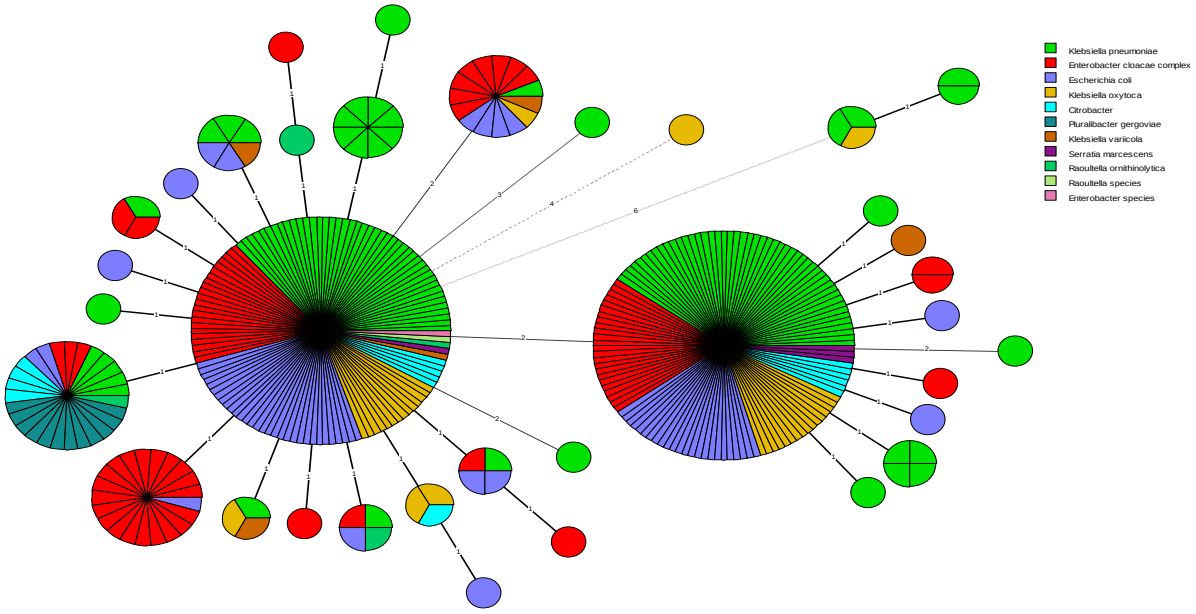


Fig.7 illustrates the relationship between IncL/M(pOXA-48) plasmid carried in different species responsible for the spread of OXA-48. The MST is based on 76 loci associated with the pOXA-48 plasmid. The MST is coloured by hospital. Two variants that differ by two alleles account for most isolates. There is a very strong association between individual hospitals and a particular variant of IncL/M. Of note some IncL/M variants have been observed exclusively or almost exclusively in one hospital. This is very persuasive evidence that OXA-48 CPE acquisition remains very strongly associated with acute hospital care and that most hospitals submitting isolates have sustained transmission though this may not always be acknowledged as a continuing outbreak. Figure 7.1 below is the same picture, coloured by species.

Figure 7.1: pOXA-48 MST – Enterobacterales – by species



Acknowledgements
We would like to acknowledge the support of the HSE, in particular Dr Philip Crowley, National Director, Quality & Patient Safety Division for their support in establishing this service. We thank Galway University Hospitals and the Saolta Group for ongoing support for the service. Thank you to the clinical laboratories who submit isolates. We would also like to acknowledge the National Reference Laboratory service of Public Health England, in particular Professor Neil Woodford, Public Health England, Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI) for advice and support in setting up the service and for their continuing support with rare or atypical isolates. We acknowledge colleagues in the Staten Serum Institute Copenhagen, Denmark for providing material for use as positive control in establishing the *mcr*-1 assay.

Martin Cormican Director of the NCPERLs

For more information please contact martin.cormican@hse.ie

Appendix 1

Total number of newly detected patients with Enterobacterales CPE per Hospital in Ireland 2018

CPE variant: / Hospital:	OXA-48	KPC	NDM	VIM	IMP	IMI	Total
The Children's Hospital Group							
Our Lady's Children's Hospital, Crumlin	3			2			**5**
Temple Street Children's University Hospital							**0**
Dublin Midlands Hospital Group							
Coombe Women and Infants University Hospital							**0**
MRH Portlaoise							**0**
MRH Tullamore	3		1				**4**
Naas General Hospital	3						**3**
St. James's Hospital	29		3				**32**
St. Luke's Radiation Oncology Network							**0**
Tallaght University Hospital	70	2	6				**78**
Ireland East Hospital Group							
Cappagh National Orthopaedic Hospital							**0**
Mater Misericordiae University Hospital	21	8	2				**31**
MRH Mullingar	2						**2**
National Maternity Hospital							**0**
Our Lady's Hospital Navan	1	1					**2**
Royal Victoria Eye and Ear Hospital							**0**
St. Columcille's Hospital							**0**
St. Luke's General Hospital Kilkenny	10						**10**
St. Michael's Hospital							**0**
St. Vincent's University Hospital	11		1				**12**
Wexford General Hospital	1						**1**
Royal College of Surgeons Ireland Hospital Group							
Beaumont Hospital	37	1	1	1		1	**41**
Cavan General Hospital	16	2					**18**
Connolly Hospital	2						**2**
Louth County Hospital							**0**
Our Lady of Lourdes Hospital	1						**1**
Rotunda Hospital	1						**1**

Saolta Hospital Group							
Galway University Hospitals	42	2	1	10		1	**56**
Letterkenny University Hospital	2		2	2			**6**
Mayo University Hospital	2			5			**7**
Portiuncula University Hospital	2						**2**
Roscommon University Hospital							**0**
Sligo University Hospital	30	1					**31**
CPE variant: **Hospital:**	**OXA-48**	**KPC**	**NDM**	**VIM**	**IMP**	**IMI**	**Total**
South/South West Hospital Group							
Bantry General Hospital							**0**
Cork University Hospital	12	7	1				**20**
Lourdes Orthopaedic Hospital Kilcreene							**0**
Mallow General Hospital			2				**2**
Mercy University Hospital	17	1					**18**
South Infirmary Victoria University Hospital	1						**1**
South Tipperary General Hospital	7						**7**
UH Kerry	1						**1**
UH Waterford	39						**39**
University Limerick Hospital Group							
Croom Orthopaedic Hospital			1				**1**
Ennis Hospital		2					**2**
Nenagh Hospital		4					**4**
St. John's Hospital Limerick		6					**6**
UH Limerick	6	38				1	**45**
Other							
Private hospitals	13	2	5		12	2	**34**
Long-termcare facilities	2						**2**
Other e.g. G.P., private laboratories	7	3					**10**
Total: (n=537)	**394**	**80**	**26**	**20**	**12**	**5**	**537**

Appendix 2

Staff of the NCPERLS

Although the NCPERLS was established with a single appointment a number of other staff in the Department of Medical Microbiology contribute to the work also to ensure continuity of service.

Elaine McGrath (Senior Scientist for NCPERLS)

Alma Tuohy

Sana Tansey

Joanne King

Niall Delappe

Mark Maguire

Wendy Brennan (Acting Senior Scientist for NCPERLS)

Maria Molloy

Tom Whyte (Chief Medical Scientist)

Anne Coleman (Quality Manager)

Belinda Hanahoe (Surveillance Scientist)

Teck Wee Boo

Deirbhile Keady

Eithne McCarthy

Una Ni Riain

Dimitar Nashev

Appendix 3

Users Guide

A copy of the recent Reference Laboratory User Guide and Request form are available through the following links:

https://saolta.ie/documents/national-carbapenemase-producing-enterobacteriales-cpe-reference-laboratory-users-guide

https://saolta.ie/documents/cpe-request-form-issue-21

http://www.saolta.ie/publications