CPEaRLS ANNUAL REPORT 2016

National Carbapenemase Producing *Enterobacteriaceae* (CPE) Reference Laboratory Service

Summary

This is the fourth annual report since the establishment of the CPE reference laboratory in late 2012. The most important point of information in this report is that in 2016 282 people were identified as colonised or infected with CPE. The total number of CPE isolates is considerably higher at 356 because a number of patients are colonised with more than one kind of CPE.

In 2016 the number of carbapenemase producing *Enterobacteriaceae* isolates increased by **156%** compared to 2015. KPC, OXA-48 and NDM remain the most common carbapenemase genes detected in Ireland. CPE was associated mainly with the species *K. pneumoniae* and OXA-48 was the most common CPE enzyme detected in 2016. Compared with 2015 there was a decrease in the number of KPC producing isolates detected; however there was a dramatic increase in the number of OXA-48 producing *Enterobacteriaceae.* This was mainly due to an outbreak in the Dublin region however OXA-48 CPE is widely distributed. 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 nursing homes is also a concern.

Figure 1: Number of Carbapenemase Producing *Enterobacteriaceae* detected in clinical samples. Sept. 2012 – Dec 2016

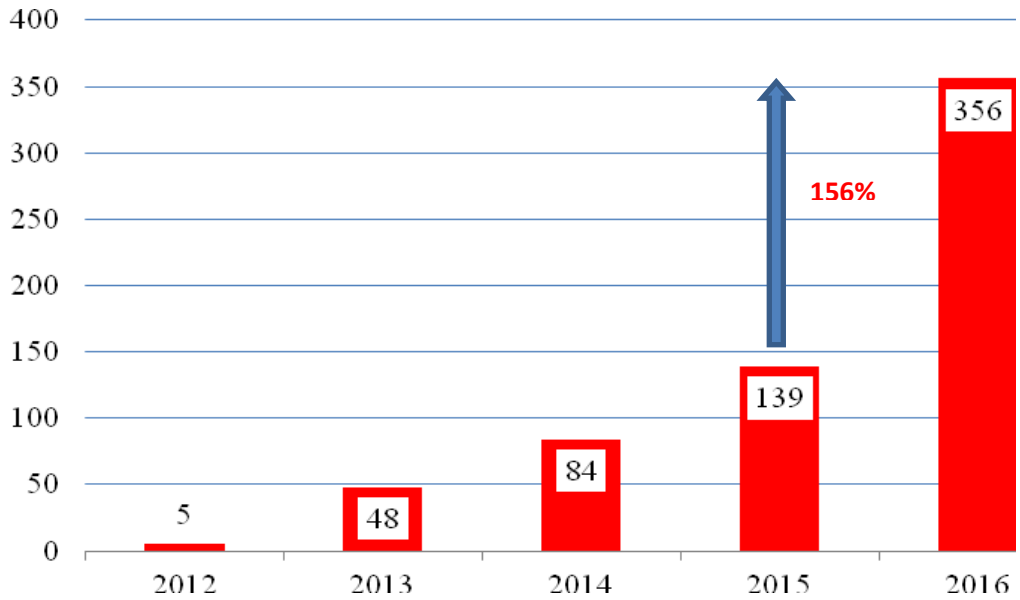


Figure 1: Carbapenemases detected in clinical isolates of *Enterobacteriaceae* in Ireland Sept 2012 to Dec 2016.

1. Establishment and Funding of the Service

The National Carbapenemase Producing Enterobacteriaceae (CPE) Reference Laboratory Service (CPERLS) was established in October 2012 by the Health Service Executive. The service is funded by a central allocation to Galway University Hospital to provide a salary for 1 Senior Medical scientist and consumable costs. The service was further supported by funding from MSD to purchase a second AB7500 Real-Time PCR System (2013) and an automated DNA extraction system (2014). This additional support has played a key role in achieving rapid turnaround times for urgent molecular testing. This represents the fourth 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 2016. The methods used in delivery of the NCPERL service are now included on the scope of accreditation of the Department of Medical Microbiology at GUH.

2. Carbapenemase Producing Enterobacteriaceae (CPE)

The website of the HSE-Health Protection Surveillance Centre (HPSC) summarises CPE Epidemiology in Ireland and contains a useful fact-sheet for patients and members of the public (http://hpsc.ie/hpsc/AZ/MicrobiologyAntimicrobialResistance/StrategyforthecontrolofAntimicrobialResistanceinIrelandSARI/CarbapenemResistantEnterobacteriaceaeCR/).
A number of policy and guidance documents related to CPE are available at www.hse.ie/hcai

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 a family known as the *Enterobacteriaceae* which includes well know bacteria such as *E. coli*, *Klebsiella pneumoniae* and *Enterobacter* spp. The *Enterobacteriaceae* are so called because they are associated with the gastrointestinal tract of humans and animals. Everyone has vast numbers of *Enterobacteriaceae* in their colon. Some *Enterobacteriaceae* cause gastrointestinal

infection (e.g. *Salmonella, Shigella*, Shiga-Toxigenic/Vero-toxigenic *E. coli*) but most *Enterobacteriaceae* (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 *Enterobacteriaceae* are important causes of blood stream infection, pneumonia and other serious infections in vulnerable groups of patients.

Antibiotics play a vital role in prevention and treatment of infection with *Enterobacteriaceae*. In recent decades many *Enterobacteriaceae* 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 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 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* increased from 10.5% (2015) to 11.1% (Q3 2016) but the percentage that were caused by multidrug resistant (MDR) *E. coli* (displaying resistance to three or more antimicrobial classes) decreased slightly to 14% (Q3 2016) from 14.6% in 2015. The figure for Carbapenemase Producing *E. coli* was stable at 0.1%. The percentage of those patients with *Klebsiella pneumoniae* bloodstream infections in which the organism was MDR was down to 7.4% in Q3 2016 from 9.8% in 2015. By Q3 2016 3 carbapenem resistant MDRKP BSI were reported compared to 7 in 2015. These 2016 data on blood stream infection suggest some reduction in numbers of cases of infection with some categories of antimicrobial resistant organism however it is not possible to determine with confidence at this point if this is a meaningful trend. It is

important to note that in 2006 only 2.5% of *E. coli* BSI were ESBL producers. It is also important to note that blood stream isolates for *Enterobacteriaceae* are the tip of an iceberg in terms of assessing dissemination of antimicrobial resistant *Enterobacteriaceae*. 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/AZ/MicrobiologyAntimicrobialResistance/EuropeanAntimicrobialResistanceSurveillanceSystemEARSS/EARSSSurveillanceReports/2014Reports/File,14686,en.pdf)

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 *Enterobacteriaceae* 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.

[Note the terms CPE and CRE are frequently used as interchangeable though this is not precise]

The Carbapenemase Producers (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 *Enterobacteriaceae* that they spread easily from person to person by the faecal-oral route directly (hand to hand) and indirectly (in water and food). 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 only one or two antibiotics available that are likely to work and in some instances there may be no entirely suitable antibiotics.

The Non-Carbapenemase Producing but Carbapenem Resistant *Enterobacteriaceae*

In some cases *Enterobacteriaceae* are resistant to carbapenems although they do not produce one of the known carbapenemase enzymes. In most cases these bacteria are resistant because of a number of smaller changes that added together make them resistant. Usually there is one or a number of changes that prevent the carbapenem from getting into the bacteria very efficiently together with one or more enzymes that are not very efficient against carbapenem but are sufficient to break down the small amount getting into the cell.

Treating Infection with Carbapenem-Resistant *Enterobacteriaceae*

Whether due to true CPE or a mixture of other changes the range of antibiotics available for treating infections with *Enterobacteriaceae* 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 *Enterobacteriaceae* 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.

When CPE cause serious infection they are an immediate threat to the life of the patient concerned. Even if the CPE are just resident in the gut it is a long-term threat to that patient, because it may subsequently cause infection. Colonisation with CPE is also a threat 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 known process that is likely to be successful at eradication. One must expect that the person who picks up CPE will remain at risk of CPE infection themselves and be a potential source of risk to others indefinitely.

Carbapenem-Resistance in Other Families of Bacteria

The situation is made more complicated because some members of certain environmental Gram-negative bacteria are naturally resistant to carbapenems examples include *Acinetobacter species* and *Stenotrophomonas maltophilia*. Other

environmental bacteria are naturally susceptible but very readily become resistant to carbapenems e.g. *Pseudomonas aeruginosa*. In some cases multi-drug resistant bacteria of these species (especially Acinetobacter species) may spread rapidly in healthcare environments but there is no indication that this is a major problem in Ireland at present

The Emergence of Transferable Colistin Resistance

For some years now one of the key treatment options for patients infected with CRE has been colistin. This is an old antibiotic that was not widely used 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 *Enterobacteriaceae* 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 in many other countries including other EU member states. The *mcr*-1 gene was not detected in any human clinical isolate in Ireland during 2016.

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 now routine in some countries. Most other systems for molecular tracking of spread of bacteria are rapidly becoming obsolete so that the transition to WGS is essential to facilitate comparability with international data. The CPE reference laboratory has made significant progresss to implement this transition.

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 sensitivity 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 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. In addition at 2 months intervals through out the year the NCPEaRLS issues by email an excel file representing an anonymised line listing of CPE detected year to date.
For information on submission of isolates please see users guide (Appendix 4).

Summary of Data for 2016

3.1 Methodology

For all isolates submitted, species identification was confirmed using MALDI-TOF. Minimum inhibitory concentration (MIC) of meropenem was performed by E-test, according to EUCAST criteria. Colistin MIC was determined on all Carbapenemase producing isolates using the TREK Sensititre (semi-automated microbroth dilution). Routine phenotypic detection and characterisation of Carbapenem resistant *Enterobacteriaceae* was determined using a commercial kit – ROSCO KPC/MBL and OXA-48 Carbapenemase Confirm Kit. To date all suspect CPE isolates submitted were analysed by molecular methods for all the more common genes associated with CPE (OXA-48, KPC, NDM-1, VIM and IMP) and a number of uncommon genes when required (IMI, GES, OXA-23, OXA-24/40, OXA-51 and OXA-58).
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 carbapenem resistance (Table 5).
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 NCPEaRLS provide the referring laboratory with a detailed report of all analyses performed for their records and with an interpretive comment where appropriate. Preliminary reports are generally provided within 7 days of receipt of isolates. 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 NCPEaRLs does not notify the isolate 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 2016

From January to December 2016 760 isolates were submitted to the NCPEaRLS by clinical laboratories throughout Ireland. This represents an increase of 87.2% on the number submitted in 2015. Managing this increase was possible because of the commitment of the medical scientists involved and the support of other staff in the Department of Medical Microbiology at GUH. Where multiple copies of an isolate were received from a given patient only the first isolate is included in the data.

Of the 760 isolates submitted a total of 679 (89.3%) displayed reduced susceptibility to one or more carbapenem antimicrobial agents by disc diffusion method- details are summarised in Table 1.

A total of 369 (48.5%) isolates were confirmed by molecular methods as carbapenemase producers - Table 2. Of the 369 carbapenemase producers 362 were *Enterobacteriaceae* isolates (Clinical *n* = 356; Environmental *n* = 6), 6 *Acinetobacter species* and 1 *Pseudomonas species*. The 356 patient isolates were from 282 patients. A number of patients were colonised with more than one kind of CPE. The number of isolates with a CPE-like phenotype exceeds the number of isolates with confirmed CPE by molecular methods. In most cases this is most likely accounted for by the known limited specificity of the phenotypic methods. It is important to note that some CPE producers, in particular *E. coli*, have ertapenem and meropenem MIC values well within the range considered susceptible for treatment purposes therefore 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. Carbapenemase producing *Enterobacteriaceae* were isolated from blood (*n* = 7), other sterile sites (*n* = 2), rectal swab/faeces (*n* = 254), urines (*n* = 54), respiratory sputum/BALs (*n* = 11), environmental samples (*n* = 6) and other sites (*n* = 28). Most isolates (64%) were from females and the age range was <1 – 93 years.

In 2016, Carbapenemase genes were associated mainly with *Klebsiella species*, with OXA-48 dominating as the most common enzyme detected followed by KPC and NDM (Table 3). There has been a dramatic increase in OXA-48, compared to figures in 2015 with a slight decrease in KPC observed (Figure 2). The increase in OXA-48 *Enterobacteriaceae* is largely due to an outbreak in a Dublin hospital - as illustrated in Table 4. KPC remains predominately associated with the University of Limerick Hospital Group.

A single isolate of *K.pneumoniae* isolated from rectal swab in the south east was found to carry 2 carbapenemase genes – OXA-48 and NDM. Co-carriage of two types of CPE was detected in 2 patients, one with OXA-48 *K.oxytoca* and KPC *K.pneumoniae* and the second with VIM *E.coli* and KPC *K.pneumoniae*. In some cases OXA-48 was identified in more than one species from a single patient.

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 5. Of the 362 CPE isolates detected 122 had additional resistance genes, mainly *bla*CTX-M group 1.

As noted in Table 6 there were 314 *Enterobacteriaceae* isolates exhibiting reduced susceptibility to one or more Carbapenem but had no CPE gene detected. These comprised of the following species *Klebsiella species* (*n* = 79), *E.coli* (*n* = 82), *Enterobacter* spp. (*n* = 133), *Citrobacter* spp. (*n* = 4) and other *Enterobacteriaceae* (*n* = 16). *Enterobacter* spp. accounted for the majority of these isolates (42.3%). High level production of ampC beta-lactamases associated with some decrease in permeability to the carbapenem antibiotics may account for the observed reduced susceptibilities in *Enterobacter* spp. Out of the 314 isolates 119 were found to have a CTX-M ESBL – results are displayed in Table 6.

During 2016, colistin MIC determined by Microbroth Dilution using the TREK Sensititre was carried out on all carbapenemase producing isolates. Isolates with a colistin MIC of ≥ 2ug /ml were further investigated by molecular methods for the presence of *mcr*-1 gene. We did not detect the *mcr*-1 gene in isolates tested during 2016.

Table 1: Summary of Isolates submitted in 2016

Species	Total No.	Meropenem		Ertapenem		Total I/R to one or more Carbapenem	No. Confirmed CPE
		S	I/R	S	I/R		
E.coli	199	163	36	36	163	163	92
Klebsiella spp	235	142	93	19	216	216	145
Enterobacter spp	212	174	38	10	202	202	74
Citrobacter spp	53	24	29	3	50	50	47
Other Enterobacteriaceae	29	27	2	9	20	20	4
Pseudomonas	20	1	19	NT	NT	19	1
Acinetobacter spp	9	3	6	NT	NT	6	6
Other Gram Negative Bacteria	3	NT	NT	NT	NT	-	0
Total	**760**						**369**

Table 2: Number of each species of bacteria submitted and key findings

Species	No. Submitted	No. with CPE Phenotype (ROSCO)	No. with Confirmed CPE	Ertapenem MIC Range CPE producers (mg/L)	Meropenem MIC Range CPE producers (mg/L)
E.coli	199	101	92	0.125 - >32	0.19 - >32
Klebsiella spp.	235	147	145	0.19 - >32	0.25 - >32
Enterobacter spp.	212	77	74	0.19 - >32	0.25 - >32
Citrobacter spp	53	47	47	0.19 - >32	0.5 - >32
Other Enterobacteriaceae	29	9	4	0.5 - >32	0.75 >32
Pseudomonas spp	20	N/A	1	NT	>32
Acinetobacter spp	9	N/A	6	NT	>32
Other Gram Negative Bacteria	3	N/A	0	NT	0.75 - >32
Total	**760**		**369**		

Table 3: Type of Carbapenemase by Species

Species	KPC	OXA-48	NDM	VIM	IMP	OXA-23	OXA-58
E.coli	4	82	5	1	0	0	0
Klebsiella spp	20	99	22	3	1	0	0
Enterobacter spp	2	66	2	4	0	0	0
Citrobacter spp	23	24	0	0	0	0	0
Other Enterobacteriaceae	1	3	0	0	0	0	0
Pseudomonas spp	0	0	0	1	0	0	0
Acinetobacter spp	0	0	0	0	0	2	4
Other Gram Negative	0	0	0	0	0	0	0
Total	**50**	**274**	**29**	**9**	**1**	**2**	**4**

Table 4: Total number of newly detected patients with *Enterobacteriaceae* CPE per Hospital in Ireland by Hospital Group 2016

Hospital Group	KPC	OXA-48	NDM	VIM	IMP
Dublin-Midlands	3	134	1	0	0
Ireland East	3	23	3	0	0
RCSI	0	7	2	0	0
University Limerick	27	1	1	0	0
South/South West	1	15	4	0	0
Saolta	6	15	12	7	1
The Children's	0	1	2	0	0
Other*	5	8	0	0	0
Total	45	204	25	7	1

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-Enterobacteriaceae 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. Comparisons between hospital groups based on these data are not valid.

Table 5: Additional Findings

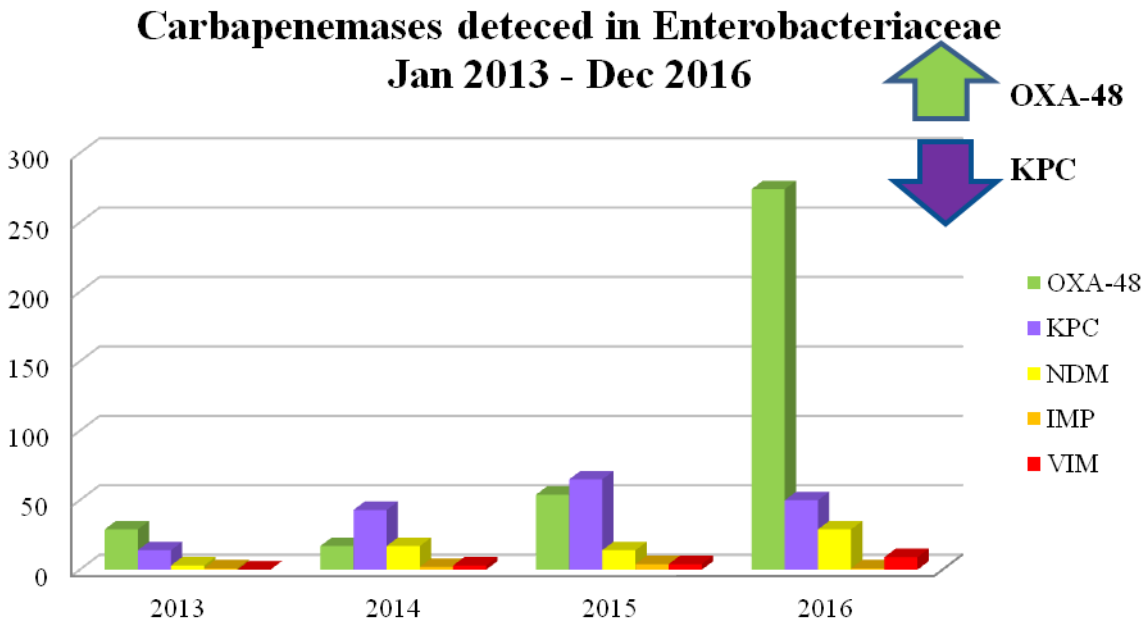
Species	CTX-M Grp 1	CTX-M Grp 2	CTX-M Grp 9	Pm-ampC
E.coli **(*n* = 199)**	66	0	8	16
Klebsiella **spp (*n* = 235)**	66	1	5	20
***Enterobacter spp* (*n* = 74)**	86	0	16	NT
Total	**218**	**1**	**29**	**26**

Note: A plasmid-mediated ampC gene together with CTX-M Group 1 was detected in 5 isolates. Two of these 5 isolates also contained an OXA-48 carbapenemase gene.

Table 6: *Enterobacteriaceae* isolates with reduced susceptibilities to Carbapenem & NO CPE gene detected

Species (I/R / Total)	**Number of isolates NO CPE gene Detected**	**No. CTX-M***	**No. Pm-ampC**
E.coli **(163/199)**	82	51 (+ampC *n* = 2)	9
Klebsiella spp **(216/235)**	79	28 (+ampC *n* = 1)	13
Enterobacter spp **(202/212)**	133	40	NT
Citrobacter spp **(50/53)**	4	0	NT
Other Enterobacteriaceae (20/29)	16	0	NT
Total	**314**	**119**	**22**

Figure 2: Carbapenemases detected in Enterobacteriaceae 2013 – 2016



Progress in 2017

For 2017 the reference laboratory service aims to continue to provide a quality service to its users and to develop capacity in relation to Whole Genome Sequencing. It is our aim to look more closely at the OXA-48 isolates and focus on the development of a specific PCR aimed at detecting the transposon we believe to be associated with the rapid spread of OXA-48 gene between species. We are also working with colleagues in UK to characterise more fully all CPE detected in Ireland to mid 2017.

In late-2017 we hope to secure an illumina MiSeq for the department which will in-turn result in improved sequencing turnaround times. In addition, it would provide an opportunity to investigate resistance and virulence genes more closely which could have an impact on understanding this public health problem.

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 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 NCPEaRLs

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Appendix 1

Total number of newly detected patients with *Enterobacteriaceae* CPE per Hospital in Ireland 2016

	KPC	OXA-48	NDM	VIM	IMP
Ireland East Hospital Group					
The Mater Misericordiae University Hospital Dublin	2	11	1		
St Vincent's University Hospital Dublin	1	6	2		
St Luke's General Hospital, Kilkenny		4			
Our Lady's Hospital, Navan		2			
RCSI Hospitals Group					
Beaumont Hospital Dublin		4	2		
Connolly Hospital Dublin		1			
Our Lady of Lourdes Hospital Drogheda		2			
Dublin Midlands Hospital Group					
St James' Hospital Dublin		4			
The Adelaide &Meath Hospital (Tallaght Hospital) Dublin	3	126			
Midlands Regional Hospital Tullamore			1		
Naas General Hospital		3			
The Coombe Women & Infant University Hospital Dublin		1			
University Limerick Hospitals Group					
University Hospital Limerick	26	1	1		
Nenagh Hospital	1				
South/South West Hospital Group					
Cork University Hospital	1	1			
University Hospital Waterford		14	4		
Saolta Hospital Group					
University Hospital Galway/Merlin Park	1	15		2	1
Letterkenny University Hospital			11		
Mayo General Hospital	5		1	5	
The Children's Hospital Group					
Our Lady's Children's Hospital, Crumlin		1	2		
Non-HSE Hospitals/Other*	5	8			
Total	45	204	25	7	1

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-Enterobacteriaceae 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. Comparisons between hospital groups based on these data are not valid. Other*: includes LTCF/Nursing homes, G.P. and private hospitals.

Appendix 2

Whole Genome Sequencing (WGS)

At the end of 2016 a selection of carbapenemase producing *K.pneumoniae* and *E.coli* were referred to UCL (University College London) Genomics Centre for WGS using the illumina NextSeq 500. Sequences were assembled using Applied Maths Bionumerics software platform (Applied-Maths, Sint-Martens-Latem, Belgium).

Phylogenetic networks were constructed (minimum spanning tree (MST) approach) based on core genome (cg)MLST using Bionumerics software– Figures 3, 4 and 5 illustrate the relationship of NDM, KPC and OXA-48 *K.pneumoniae* respectively and Figure 6 illustrates a minimum spanning tree for OXA-48 *E.coli*. Phylogenetic networks are based on the comparison of allelic profiles using distance matrices corresponding to the percentage of distinct loci. 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.

Sequences were also analysed using ResFinder (Centre for Genomic Epidemiology: https://cge.cbs.dtu.dk/services/ResFinder/): which identifies acquired resistance genes in sequenced isolates of bacteria. All large volume of information is generated from ResFinder including Sequence Type (ST), Virulence Genes, Resistance genes and Plasmids. Figures 7 and 8 are screen shots highlighting the volume of information generated from the ResFinder database. We are continuing to explore how to apply this technology to form a better understanding of pathways of spread.

Figure 3: MST – cgMST NDM *K.pneumoniae* 2016

This figure shows that groups of NDM 1 *K. pneumoniae* from a given hospital laboratory are often very closely related which is consistent with local networks of transmission.

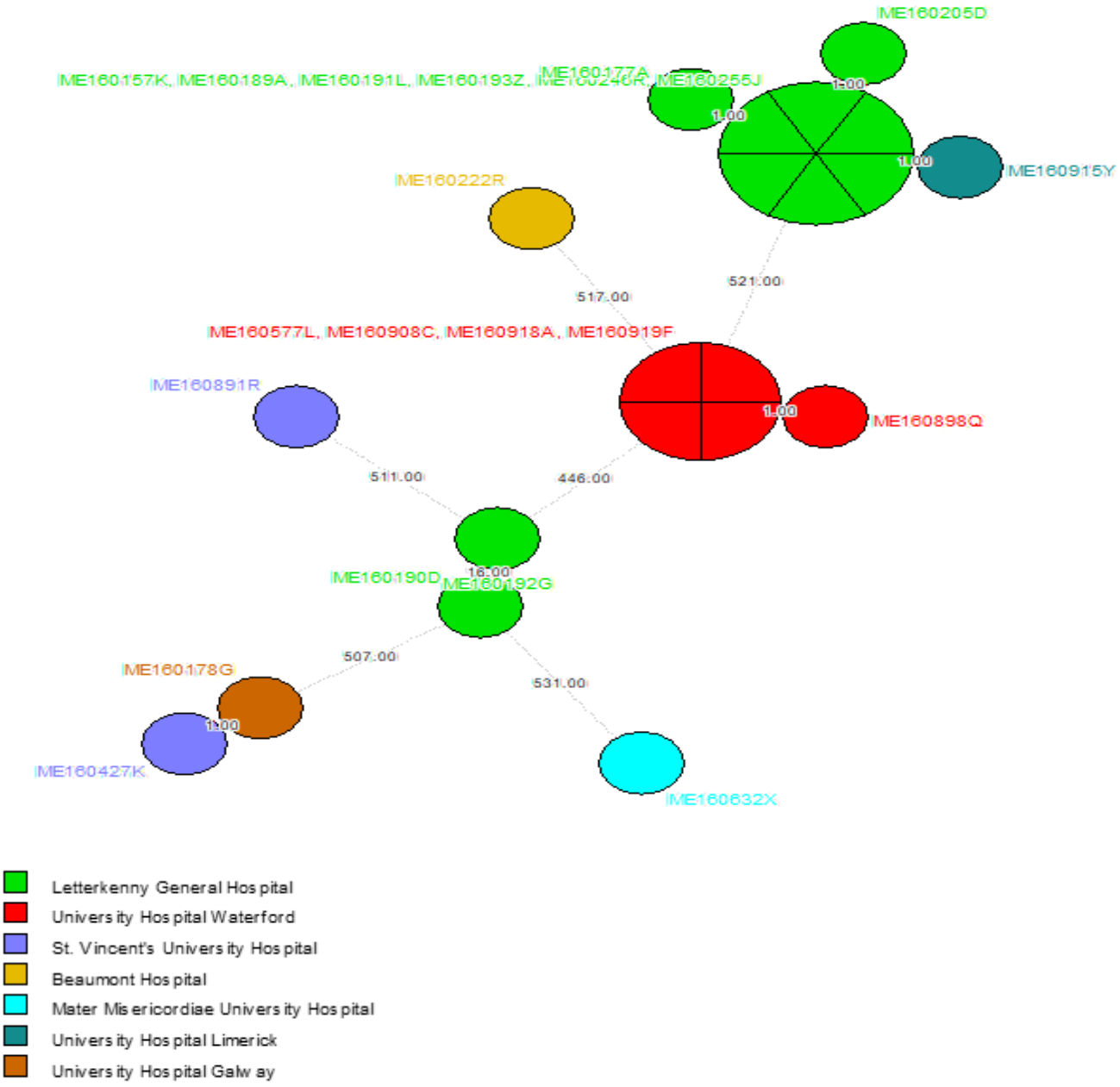


Figure 3 illustrates the phylogenetic network for NDM *K.pneumoniae*, labelled by referring lab. A total of ten isolates (labelled **green**) were received from the laboratory in Letterkenny University Hospital were NDM and CTX-M Group 1. These isolates were not from patients in that hosptial but from another hospital served by that laboratory. Eight of the isolates formed a single clonal group (≤1 allele difference) - sequenced as ST-111. Two of the 10 isolates are quite distinct between from the other 8 but very similar to each other (ST36). This most likley represents plasmid transfer between the ST11 group and the ST36 group and subsequent onward transmission.

One isolate identified submitted from from University Hospital Limerick (ME160915) is very similar to the ST11 group suggesting a link between this patient and the outbreak group.

A cluser of 5 NDM and CTX-M Group 9 *K.pneumoniae* isolates was identified at University Hospital Waterford, labelled in **red**. One isolate ME160898 had one allele difference and was found to have an additional plasmid carrying OXA-48. There is no thus evidence at this level of a link between the NW and SE outbreaks although further work to analyse for plasmid transfer may be informative.

Figure 4: MST – cgMLST KPC *K.pneumoniae* 2016

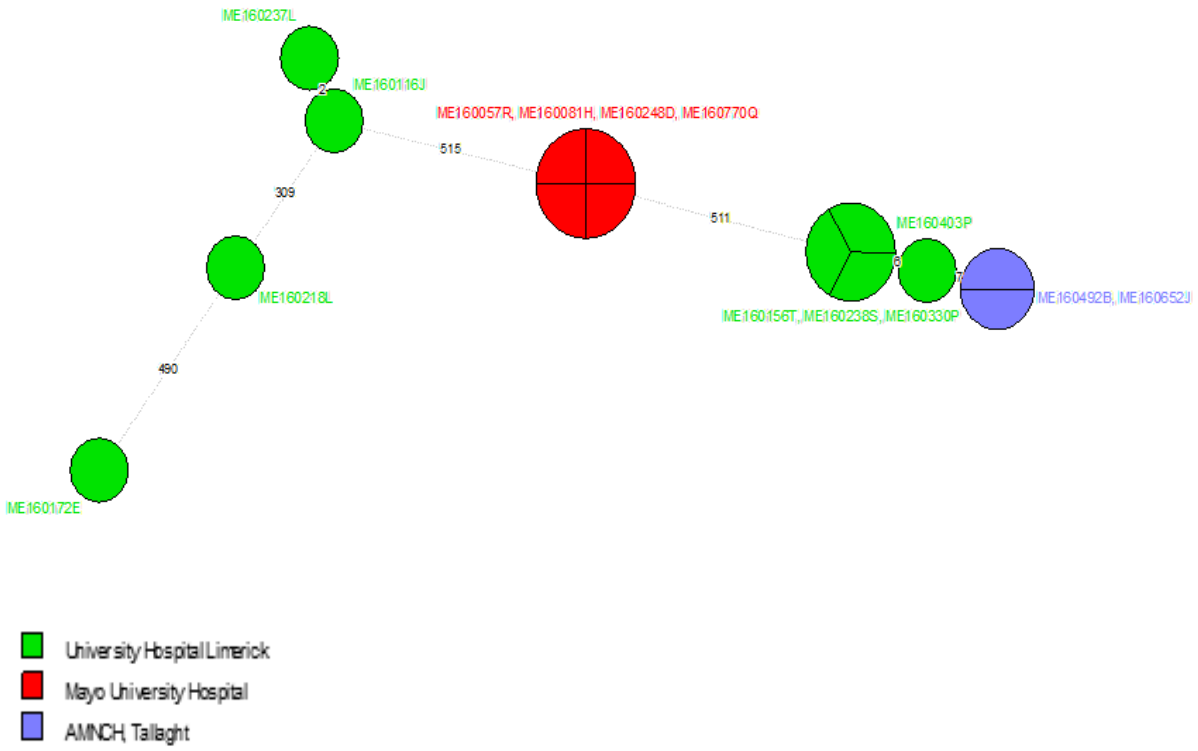


Figure 4 above illustrates the relationship between KPC producing *K.pneumoniae* isolates sequenced in 2016. Labelled in **red** is an indistinguishable cluster of ST-323 *K.pneumoniae* identified by the laboratory at Mayo University Hospital. The University Hospital in Limerick (labelled **green**) accounts for the majority of KPC isolates identified to date. Of the select few isolates of *K.pneumoniae* sequenced in 2016 5 distinct Sequence Types were identified (ST-26, ST-27, ST-258, ST-512, ST-922) and 2 variants of KPC (KPC-2 and KPC-3). This picture is consistent with a discrete temporal outbreak in Mayo University Hospital during which time little diversity emerged. The data from the laboratory at University Hospital Limerick suggests that there are two events happening one related to KPC 2 and one related to KPC 3. KPC has been endemic in the Mid-West area for some years and the diversity observed among these isolates most likely reflects plasmid dissemination to multiple *K. pneumoniae* clonal groups over time.

Figure 5: MST cgMLST OXA-48 *K.pneumoniae* 2016

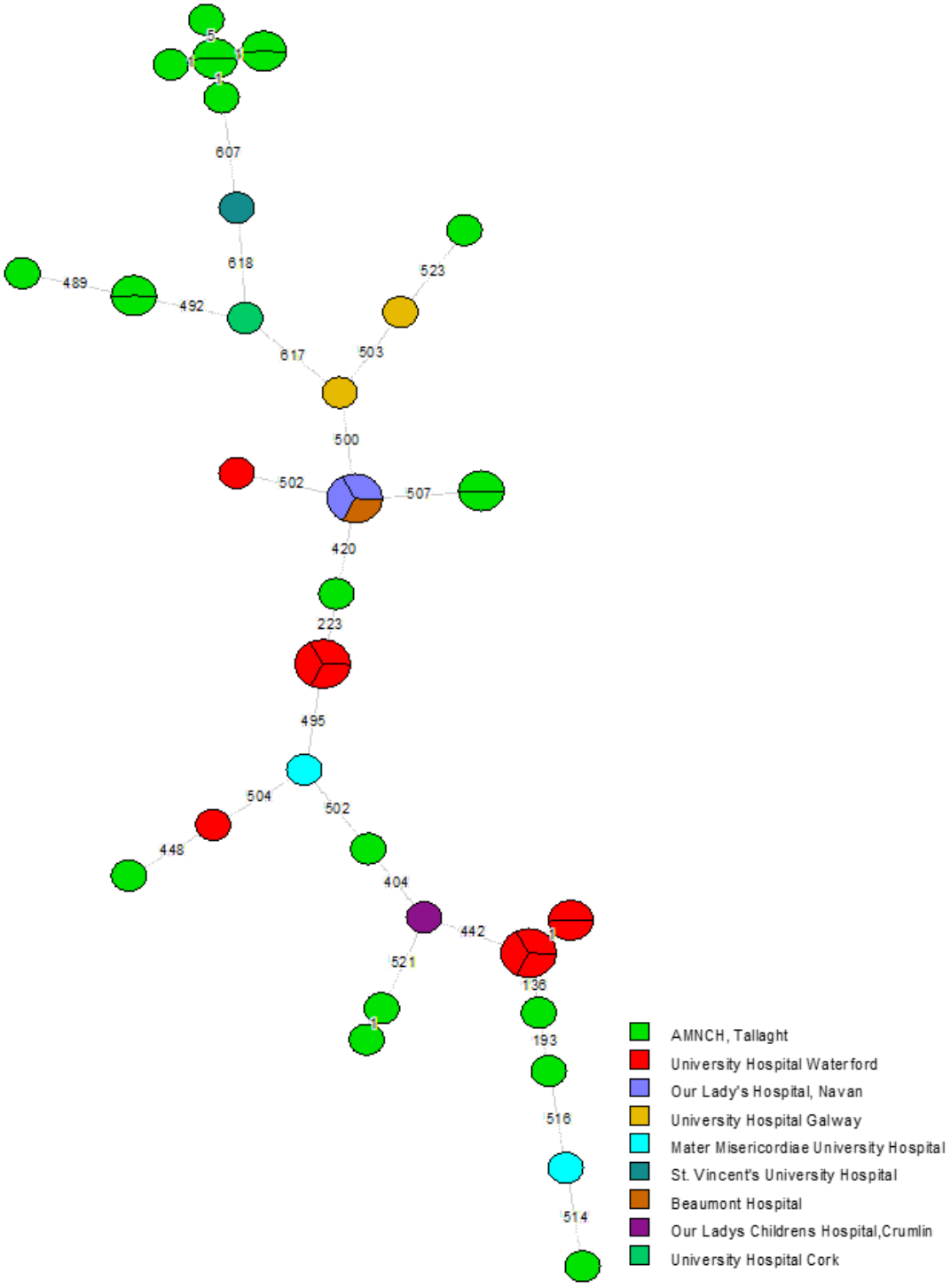


Figure 5 illustrates the phylogenetic relationship between the OXA-48 *K.pneumoniae* isolates sequenced in 2016. With the exception of a select few clonal clusters identified the vast majority of OXA-48 *K.pneumoniae* isolates are genetically diverse. Sequencing identified a total of 21 Sequence Types. The AMNCH, Tallaght hospital outbreak was associated with 11 different ST types, however sequencing revealed a common plasmid amongst these strains – IncL/M(pOXA-48).

Figure 6: MST cgMLST OXA-48 *E.coli* 2016

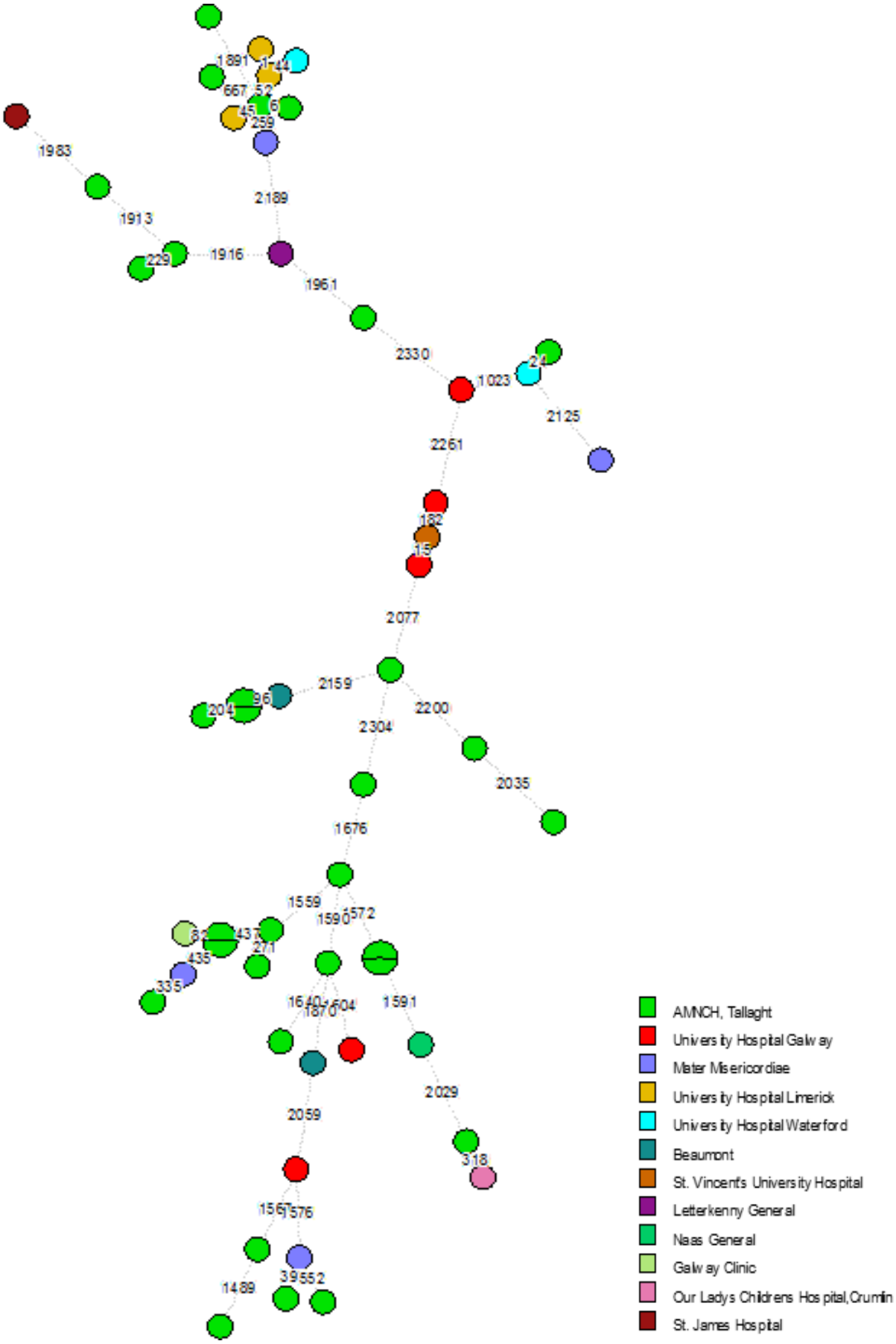


Figure 6 above illustrates the MST based on cgMLST for OXA-48 *E.coli* isolates sequenced in 2016. AMNCH, Tallaght accounted for the majority of OXA-48 *E.coli* isolates in 2016 (coloured **green**). Even though there was a large hospital outbreak, similar to the phylogenetic network of OXA-48 *K.pneumoniae* in Figure 5, it is clear that the outbreak involved a very large number of clonal groups of *E. coli*. Further analysis suggest that this outbreak is related to a particular plasmid – IncL/M(pOXA-48) that disseminates so rapidly between different variants of *E. coli* and between different species of *Enterobacteriaceae* that typing based on the organism carrying the plasmid is largely irrelevant to tracking spread .

Figure 7: Screen Shot 1 from ResFinder Database

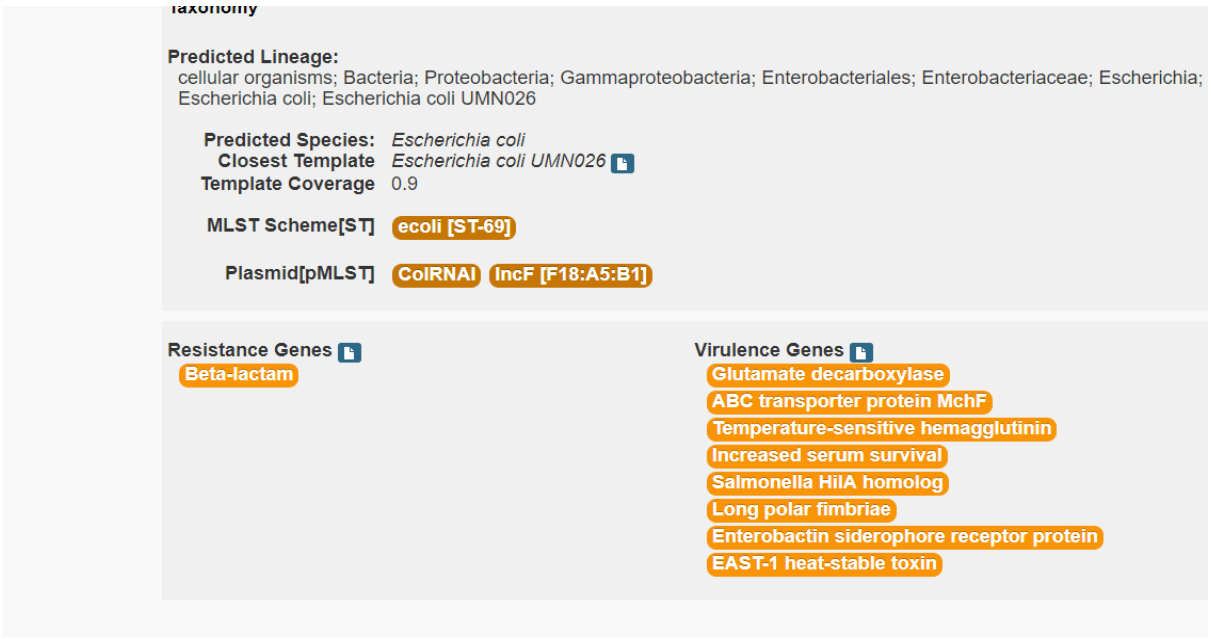
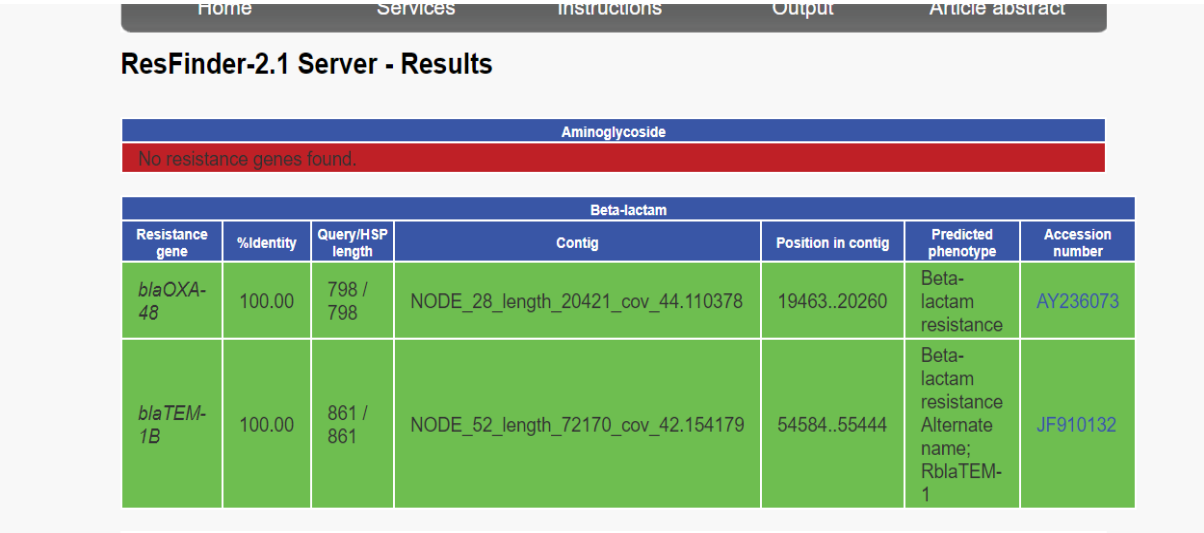


Figure 7 above illustrates information generated for an OXA-48 *E.coli* sequenced. Information includes Sequence Type, Resistance and Virulence Genes.

Figure 8: Screen Shot 2 from ResFinder Database



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ResFinder-2.1 Server - Results

Aminoglycoside
No resistance genes found.

Beta-lactam						
Resistance gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted phenotype	Accession number
blaOXA-48	100.00	798 / 798	NODE_28_length_20421_cov_44.110378	19463..20260	Beta-lactam resistance	AY236073
blaTEM-1B	100.00	861 / 861	NODE_52_length_72170_cov_42.154179	54584..55444	Beta-lactam resistance Alternate name; RblaTEM-1	JF910132

Figure 8 above provides more detailed information on the Resistance Genes detected including Contig location and predicted phenotype.

Appendix 3

Staff of the NCPEaRLS

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)

Sana Tansey

Joanne King

Maeve Hetherington

Wendy Brennan

Tom Whyte (Chief Medical Scientist)

Frances Higgins/Anne Coleman (Quality Manager)

Belinda Hanahoe (Surveillance Scientist)

Teck Wee Boo

Deirbhile Keady

Eithne McCarthy

Marianne Nolan

Una Ni Riain

Dimitar Nashev

Dearbhaile Morris (NUIGalway)

Appendix 4

Users Guide

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

http://www.saolta.ie/publications

Appendix 5 Associated Presentations and Publications

TW Boo, N O'Connell, J King, McGrath E, R Hill. First report of IMI carbapenemase-producing colistin-resistant *Enterobacter* clinical isolate in Ireland. Euro Surveill. 2013; 18 (31)

Prof. Hajo Grundmann *et al*. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. The Lancet: Infectious Diseases. 2017; 17 (2): 153 – 163

O'Connor, Ciara; Cormican, Martin; Wee Boo, Teck; McGrath, Elaine; Slevin, Barbara; O'Gorman, Alan; Commane, Marion; Mahony, Stephane; O'Donovan, Eimear; Powell, James; Monahan, Regina; Finnegan, Cathriona; Kiernan, Miranda G; Coffey, Calvin J; Power, Lorraine; O'Connell, Nuala H; Dunne, Colum. An Irish outbreak of New Delhi metallo-β-lactamase (NDM)-1 carbapenemase-producing Enterobacteriaceae: increasing but unrecognised prevalence. 2016; 94 (4): 351 - 357