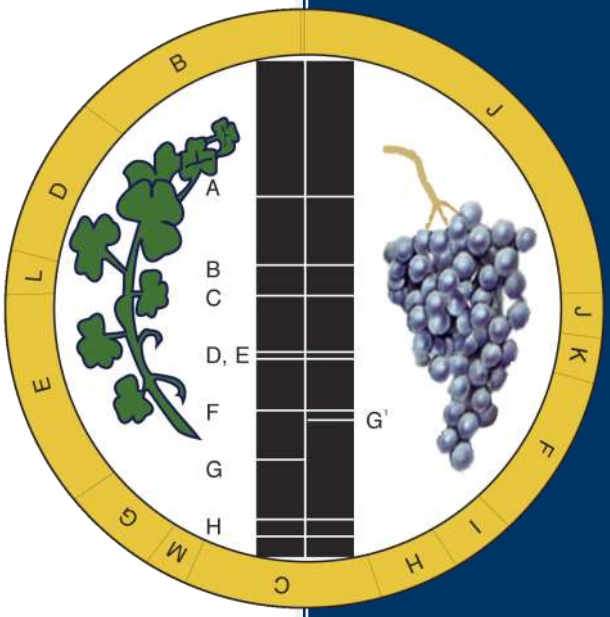
ANNUAL REPORT **2017**



National Meticillin-Resistant *Staphylococcus aureus* Reference Laboratory

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INTRODUCTION

This annual report details the work of the National MRSA Reference Laboratory in 2017. Throughout 2017, the laboratory continued to deliver on its role in assisting medical professionals is the control of MRSA in hospitals and the community in Ireland.

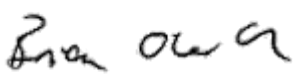
In addition to the routine work of the laboratory which includes molecular typing for epidemiological investigation and the detection of important virulence factors, service developments and projects during the year included:

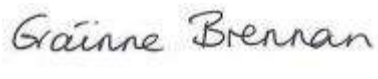
- the expansion of molecular tests to investigate linezolid resistance in coagulase negative staphylococci (CoNS) and enterococci;
- further characterisation of community associated (CA) and livestock associated (LA)- MRSA and Meticillin susceptible *S. aureus* (MSSA) recovered in Ireland using DNA microarray;
- Characterisation of *S. aureus* isolates recovered from healthcare workers, patients and their environment;
- the staff of the laboratory continued to provide education and training to doctors, nurses and scientists and contribute to MRSA research by completing/collaborating in numerous publications.

The laboratory also began evaluating ways in which whole genome sequencing may provide users of the laboratory with assistance when investigating outbreaks caused by MRSA. To date several outbreak incidents have been investigated using this technology and the laboratory hopes to further expand this service in the near future.

We would like to thank the staff of NMRSARL who continue to work tirelessly to provide the service; our collaborators in research and development which yields a fruitful new knowledge on MRSA and the Laboratory Medicine Directorate and St. James's Hospital for continuing to support the NMRSARL in the important work that it provides.

We hope that you find the following pages useful and informative.





Dr. Brian O'Connell
Director

Dr. Gráinne Brennan
Chief Medical Scientist

SUMMARY

Public health impact

- The surveillance and identification of potential outbreaks of MRSA, MSSA and CoNS
- The laboratory also monitors the incidence of *pvl* carrying strains of *S. aureus* and the strains associated with healthcare infections

New service developments

- Phenotypic and genotypic methods remain under constant review to take advantage of any newly developed methodologies;
- Investigation of linezolid resistance among enterococci and CoNS

Activity

- During 2017, the EARS-Net project accounted for 20% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for 35.2%
- Further increase in the uptake of newer services including DNA microarray profiling of *S. aureus* and investigation of linezolid resistance among Enterococci and CoNS

Research and development

- The laboratory continues to participate in numerous projects and is collaborating with the Dublin Dental University Hospital, Trinity College to evaluate the potential of whole genome sequencing for routine use

Education and training

- The laboratory continues to offer training to biomedical science students and postgraduate students in Trinity College Dublin and Dublin Institute of Technology
- The Chief Medical Scientist completed a PhD investigating emerging strains of MRSA in the community, among livestock and in healthcare facilities in Ireland

Future developments

- As technology expands into whole genome sequencing, this technology will replace a number of the current assays and produce definitive data on the similarities and differences between organisms

ROLE OF THE LABORATORY

Since its establishment in 2002, the Laboratory has supported efforts to prevent and control MRSA in Ireland by providing expertise to laboratories in the correct identification of *Staphylococcus aureus* isolates, by tracking circulating strains as part of infection control, by detecting the emergence of new mechanisms of resistance to antibiotics, by screening for the presence of novel virulence factors or toxins, and by participation in research and development initiatives at home and abroad.

SERVICES

The NMRSARL provides the following services:

- Investigation of MRSA isolates using phenotypic and molecular techniques for the following reasons:
  - confirmation of *S. aureus* identity
  - epidemiological typing (including *spa* typing)
  - detection of resistance and virulence genes including *pvl, mec, nuc, eta, etb* and *etd*
- Investigation of meticillin susceptible *S. aureus* (MSSA) isolates
  - For the detection of the *pvl* and exfoliative toxin genes
  - Outbreak investigation of strains using *spa* typing
- Advice
  - on treatment and management of patients with MRSA through its medical director
  - on infection control through the infection control team of SJH
  - on laboratory aspects of MRSA through the scientific staff of the laboratory

ISOLATES

Isolates, recovered from patients attending community medical practitioners or hospitals, are submitted to the laboratory from all hospital microbiology laboratories throughout the Republic of Ireland.

In addition to this the NMRSARL also provides laboratory support for the MRSA component of EARS-Net in Ireland. All Irish hospital laboratories participating in EARS-Net send MRSA isolates from blood cultures (one per patient per quarter) to NMRSARL where they are investigated for resistance to oxacillin, vancomycin and teicoplanin using standard E-test or E-test™ macro-method techniques. NMRSARL also provides data on rates of resistance to other clinically useful antibiotics.

PUBLIC HEALTH IMPACT

The impact of the various activities of the NMRSARL on public health is described below.

Organism	Activity	Number of isolates	Outcome
MRSA blood culture isolates	Surveillance	162	Participation in EARS-Net which is a European wide network of national surveillance systems, providing European data on antimicrobial resistance for public health purposes
MRSA & MSSA	PVL toxin testing	418	Surveillance, recognition, investigation and management of PVL *S. aureus* in Ireland
MRSA & MSSA	Surveillance analysis and identification of trends	828	Typing and susceptibility testing of MRSA and MSSA isolates submitted throughout the year.
ST1-t127-MRSA-IV	Surveillance	47	Investigation of isolates recovered from community and healthcare sources between 2013 and 2016 in order to investigate the isolate relationships and the extent of their spread
Mupirocin resistant t127-MRSA-IV	Surveillance	27	Ongoing surveillance of mulitantibiotic resistant strain which was initially limited to one hospital but which has since spread to other hospitals and the community
MRSA and MSSA	Surveillance	210	Outbreak (n=62) investigations throughout Ireland
MRSA and MSSA	Confirmation of resistance against various antibiotic agents	439	Confirmation of resistance against glycopeptides, β-lactams, daptomycin and newer agents.
VRE and CoNS	Confirmation of linezolid resistance	76	Characterisation of resistance mechanism associated with increased linezolid resistance in VRE and CoNS
MSSA & MRSA	Characterisation of *S. aureus* recovered from healthcare workers	370	Determine prevalence of *S. aureus* among closed communities in Ireland. Isolates were recovered from prisoners in a prison in the midlands.
LA-MRSA	Surveillance	7	Characterisation of MRSA strains recovered from humans but with known association to livestock including isolates harbouring the *mecC* gene.

REFERENCE LABORATORY WORK

During 2017, work under the EARS-Net project accounted for 20% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for 35.2% (Fig. 1) In recent years an increase in requests for investigations of MSSA isolates has led to a change in the services of the laboratory and 2017 saw a further increase in the uptake of newer services including DNA microarray profiling of *S. aureus* and investigation of linezolid resistance among Enterococci and CoNS.

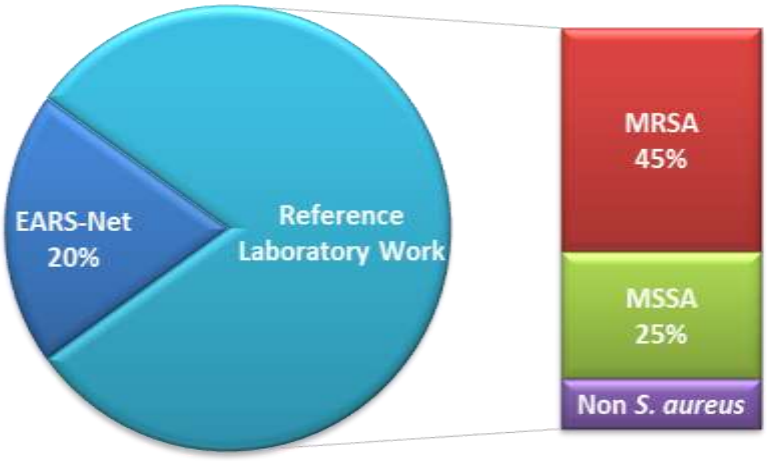


Figure 1. Workload of the NMRSARL during 2017

Along with a steady increase in the number of isolates submitted, the complexity of tests has also increased over time. Currently the laboratory performs phenotypic investigation on all isolates submitted however further molecular investigation is performed on over half of the isolates including investigation for PVL toxin (n=418) or *spa* typing (n=316). This change is primarily due to the changing epidemiology of MRSA circulating in Ireland and the limited information that can be obtained from phenotypic investigation of these emerging strains.

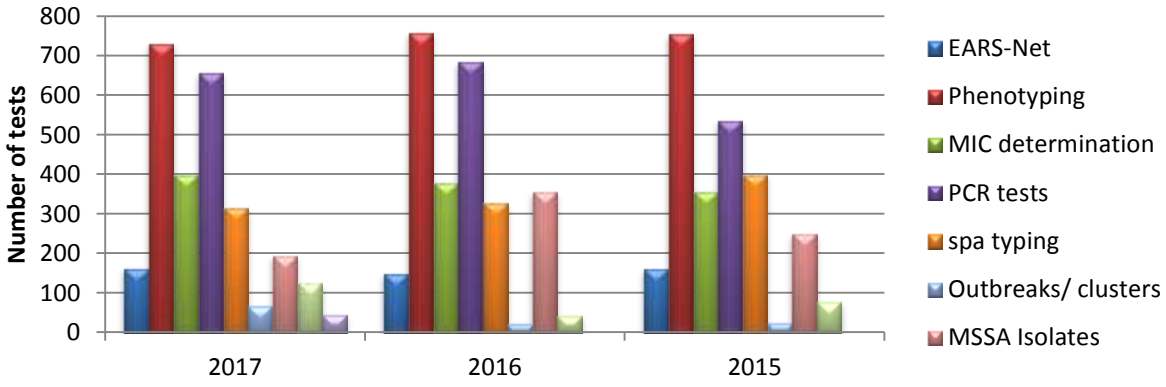


Figure 2. Distribution of workload throughout 2017

Linezolid resistance in Staphylococci and Enterococci

In response to the HPSC alert of May 2016 regarding linezolid resistance in Ireland, the NMRSARL expanded the testing repertoire to include *cfr* and *optrA* gene detection in coagulase negative staphylococci and Enterococci (1).

Linezolid is often the drug of last resort to treat serious infections caused by Gram-positive cocci. While resistance frequently arises due to mutations in the 23S rRNA gene, altering the drug binding site, and/or the 50S ribosomal proteins L3, L4 and L22, impairing linezolid binding, less frequently it has also been associated with the acquisition of a plasmid-encoded methyltransferase gene *cfr* or ABC transporter gene *optrA.* The presence of *cfr* can result in the PhLOPS$_A$ phenotype i.e., resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A compounds, due to their overlapping binding sites. In contrast however, *optrA* confers resistance to oxazolidinones and phenicols only.

In 2017 the NMRSARL was involved in a research project with collaborators based in the Dublin Dental University Hospital investigating an outbreak of linezolid resistant *S. epidermidis* and found that an *E. faecium* harbouring both *cfr* and *optrA* which at the time of publication was the only report of a single isolate harbouring both resistance genes (2). More recently however another resistance gene, *poxtA* has been associated with linezolid resistance. In addition to linezolid and phenicol resistance this too encodes resistance to tetracyclines and has been recognised in an Irish isolate which also harbours *optrA*. The NMRSARL are currently validating a PCR assay to detect all three genes.

While the numbers investigated to date remain low, since introduction approximately 8% of isolates investigated have been found to harbor the *optrA* gene. However while in 2016 a near equal proportion of *E. faecalis* and *E. faecium* were found to harbor the gene, in 2017 *optrA* was detected in only a single *E. faecium* with the remaining positive isolates identified as *E. faecalis* (Fig. 3). Isolates exhibiting phenotypic resistance but lacking the *cfr* or *optrA* genes were not further investigated for resistance due to mutations.

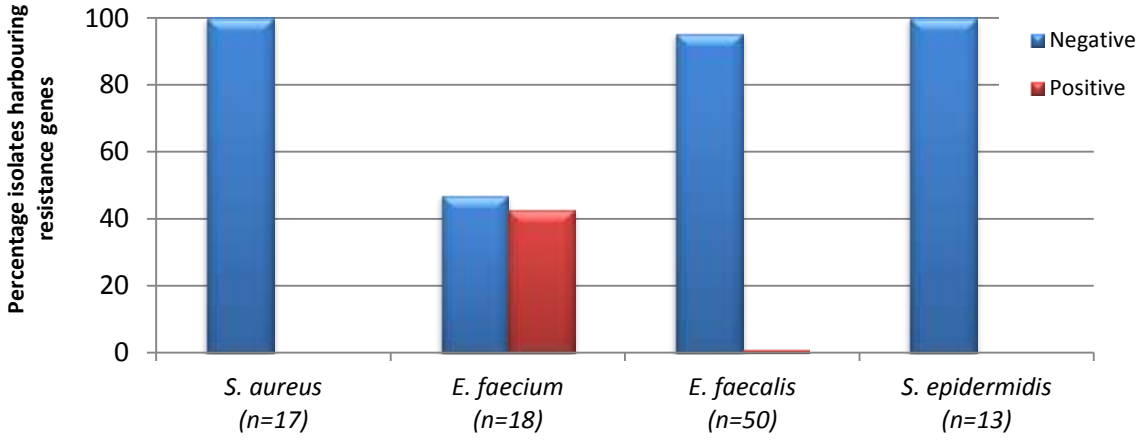


Figure 3. Distribution of linezolid resistant isolates investigated in 2017. While *E. faecalis* accounted for the majority of isolates tested, only one was *optrA* positive while 42.9% of *E. faecium* isolates investigated were found to be positive.

PVL positive *S. aureus*

Throughout 2017 the detection of PVL continued to be the most frequently requested test. The PVL toxin is a cyto-toxigenic toxin produced by *S. aureus* which is clinically associated with skin and soft tissue infections but is rarely reported in isolates recovered from invasive infections. In 2017, 418 *S. aureus* isolates were tested for carriage of the *lukS-PV* and *lukF-PV* genes encoding for PVL comprising of 246 MRSA and 172 MSSA.

Among the MRSA isolates 20% (47/246) were found to be positive while 10% (17/172) of MSSA isolates were also positive.

The PVL-positive MRSA population continues to be less diverse with 50% of the isolates associated with only three types (ST8, 28%; ST30, 15%; ST772, 9%) [Fig. 4].

Among the isolates recovered from blood stream infections only 3% were PVL positive (5/162) however all of these were assigned to ST8. Also known as USA 300, has been recognised among Irish PVL positive isolates since 2007 and has since then been one of the most frequently recognised strains every year. Although associated with the community here in Ireland, in the USA this strain has caused a number of prolonged nosocomial outbreaks and has established itself as the predominant strain in American health care facilities.

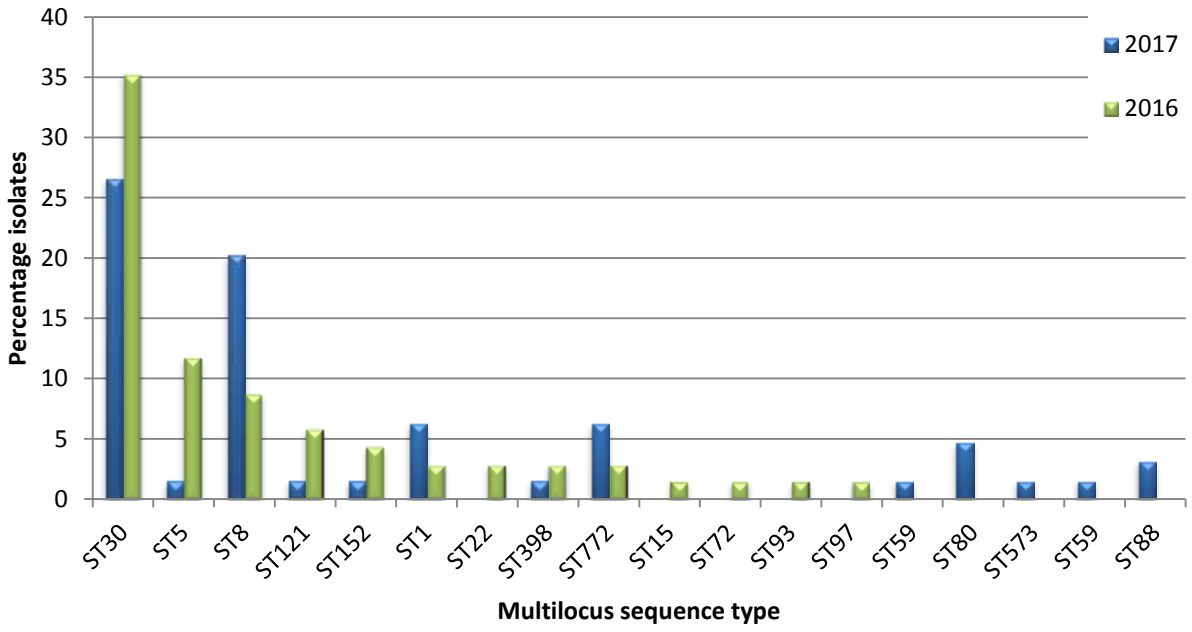


Figure 4. Multilocus sequence types of PVL positive *S. aureus* isolates recovered throughout 2017

ANTIMICROBIAL RESISTANCE AMONG MRSA IN IRELAND

The phenotypic epidemiological typing techniques used in the NMRSARL enables the laboratory to monitor resistance among MRSA strains against clinically useful antimicrobial agents and to identify emerging resistance that may cause concern into the future with the EARS-Net isolates providing a representative collection of isolates recovered throughout the country. The current predominant strains circulating in Ireland (ST22-MRSA-IV) exhibits a non-multiantibiotic resistant susceptibility profile, emerging community associated strains however carry multiple virulence and resistance genes.

While resistance to macrolides, fusidic acid and ciprofloxacin is common in the ST22-MRSA-IV strain in Ireland, in 2017 there was an increase in aminoglycoside and tetracycline resistance detected among isolates recovered from blood stream infections (Fig. 5). Often these resistance profiles are associated with strains circulating in the community.

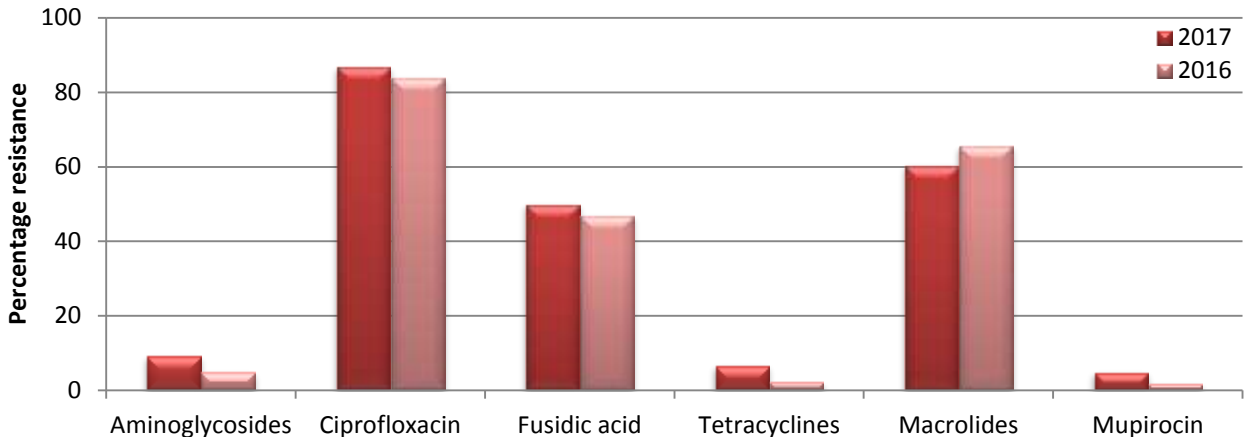


Figure 5. Resistance rates among EARS-Net isolates recovered in 2017

The increase in resistance to fusidic acid is a trend which has been observed over a number of years having increased from 27% in 2006 to the current level of 50% and is a worrying concern since fusidic acid (FA) remains a clinically useful antimicrobial for difficult to treat skin and soft tissue infections.

Among the FA-resistant isolates, 82.5% are ST22-MRSA-IV and resistance among these isolates is predominantly associated with mutations in the *fusA* gene. Resistance among the remaining isolates is associated with the *fusC* gene.

The *fusC* gene is located either (i) within a SCC element, either alone or adjacent to a SCC*mec* element where it forms part of a composite island (CI), or (ii) within a SCC*mec* element, where it is termed a chimeric element due to the presence of *mecA* and *fusC* within a single SCC*mec* element.

Work in the NMRSARL has found extensive genetic diversity among *fusC*-positive MRSA isolates in relation to their genetic backgrounds and the SCC*mec*-*fusC* elements that they harboured and the NMRSARL is currently collaborating with colleagues in the Dublin Dental University Hospital to further investigate these isolates.

Antimicrobial susceptibility among MRSA recovered from non- blood stream infections

While the previously mentioned rates of resistance relate only to EARS-Net isolates, a greater proportion of the work in the NMRSARL relates to isolates recovered from non-blood stream infections. In addition these isolates are often recovered from patients in the community where no risk factors for MRSA infection are present.

These isolates are submitted from different users on an ad hoc basis and therefore do not represent true prevalence characteristics of strains in the community. However it is possible to determine resistance profile of the isolates that were selected for submission to the NMRSARL.

Below shows the profile of all non-BSI isolates investigated in comparison to those of BSI isolates. Typically in Ireland ST22-MRSA-IV is the predominant HA-MRSA accounting for 80% of MRSA investigated under the EARS-Net project and exhibits a non-multiantibiotic resistant profile. However the non-BSI isolates recovered both in healthcare facilities and in the community, and which may also be among others, ST22-MRSA-IV, exhibit higher levels of resistance against the panel of antibiotics tested with 84% of isolates exhibiting multi-antibiotic resistance, that is, resistance to three or more different classes of antibiotics and in particular to aminoglycosides, mupirocin and tetracycline.

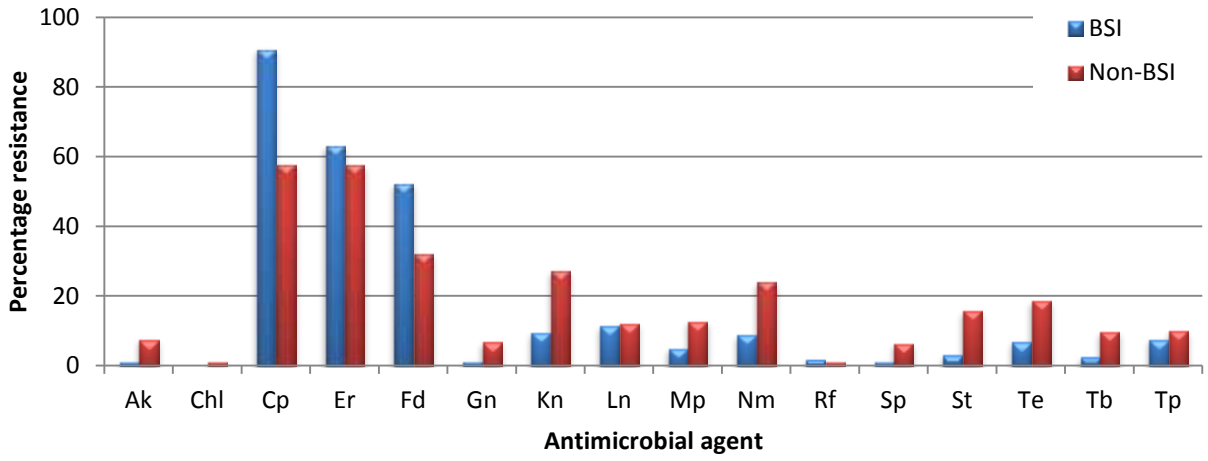


Figure 6. The percentage of blood stream MRSA isolates exhibiting resistance to each antimicrobial agent in comparison to those recovered from non-blood stream infections.

Resistance patterns determined for MRSA isolates by antibiogram- resistogram typing. Percentage for each agent includes those exhibiting both resistance and intermediate resistance as determined in accordance with EUCAST or in-house developed interpretive criteria. Abbreviations: Ak; amikacin, Chl; chloramphenicol, Cp; ciprofloxacin, Er; erythromycin, Fd; fusidic acid, Gn; gentamicin, Kn; kanamycin, Mp; mupirocin, Nm; neomycin, Sp; spectinomycin, St; streptomycin, Te; tetracycline, Tb; tobramycin, Tp; trimethoprim.

ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND

Like Europe, ST22-MRSA-IV is the pandemic clone in Ireland and, in 2017, was associated with 76.4% of MRSA causing blood stream infections. This strain is known also known as UK-EMRSA-15, Barnim Epidemic Strain, Spanish PFGE type E13, or Canadian MRSA-8 (3)

This strain has been reported in many countries and, where present, tends to be the predominate strain accounting for >50% of MRSA in Portugal, and Malta and in England it is currently associated with 85% of bacteraemia cases. The strain occurs in hospitals as well as among outpatients in the community but it has also been recovered from companion animals such as horses, cats and dogs (3).

Common resistance patterns exhibited by the ST22-MRSA-IV strain include resistance to fusidic acid, ciprofloxacin, and erythromycin. Variable virulence markers in ST22-MRSA-IV are *sec* and *sel* as well as the IEC genes encoded by lysogenic β-haemolysin-converting phages (*sak, chp, scn*) (3).

In Ireland in 2017 *spa* type t032 continued to be the most predominant strain among ST22 MRSA isolates however other ST22 associated *spa* types including t022, t515, t223, t2945 and t020 were also recognised.

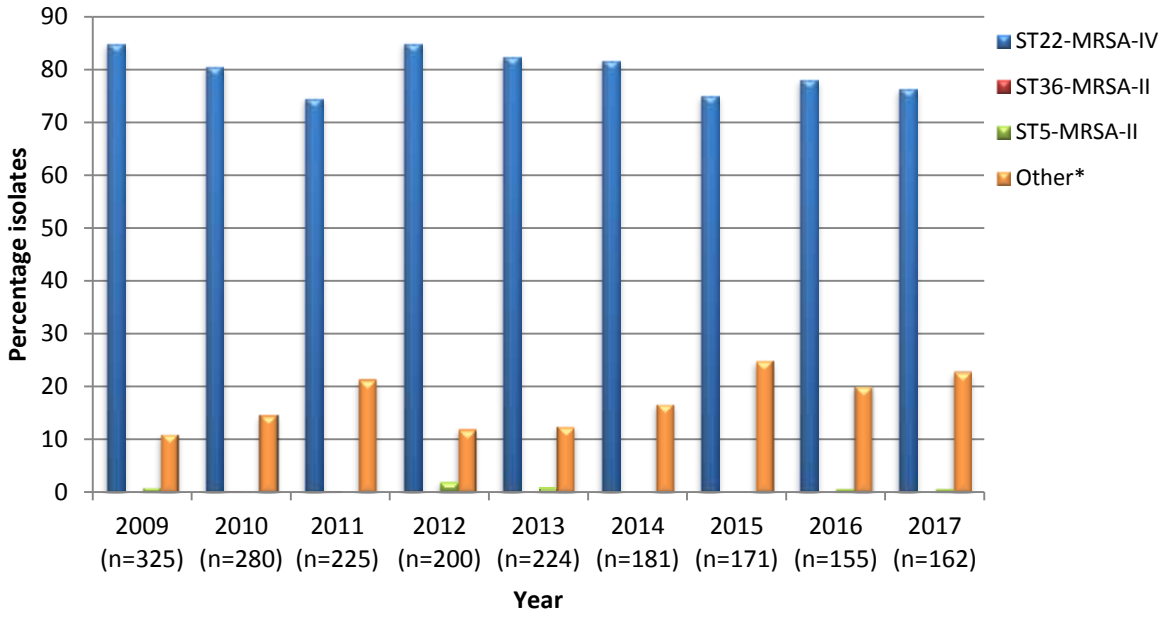


Figure 7. Epidemiological types of MRSA strains recovered from blood stream infections inferred using antibiogram resistogram (AR) typing during 2017. During the year historical AR types AR13 and AR14 (both associated with ST8-MRSA-II) were not detected among EARS-Net isolates. The total number of isolates investigated each year is shown in parentheses.

***Further molecular analysis of isolates categorized as 'other' assigned isolates to numerous sequence types however predominant STs included ST1, ST5 and ST8 all carrying SCC*mec*IV.**

MOLECULAR EPIDEMIOLOGICAL TYPING OF MRSA

Typing methods for discriminating different bacterial isolates are essential epidemiological tools in infection prevention and control. Traditional methods based on phenotypic characteristics have been used for many years however often fail to provide sufficient discrimination of isolates in outbreak situations. In addition, the acquisition of other resistance mechanisms, along with the emergence of newer MRSA strains has led the NMRSARL to explore other typing methods to allow easier comparison of MRSA recovered in Ireland.

spa typing involves sequencing of the Staphylococcal protein A gene (*spa)* to recognise mutations or repeat insertion/deletion events that can cause changes in the polymorphic X region of the *spa* gene. It has become a well-established discriminatory method for outbreak investigations but has also been shown to be useful for long-term epidemiological studies. The availability of MLST data associated with *spa* types on an online database facilitates comparison of Irish isolates with isolates from all other countries. Based upon repeating patterns (BURP) analysis clusters *spa* types together based on the repeat succession pattern of *spa* types (4) .

Using the inferred MLST data available from the *spa* typing online database the most frequently recognised MLST types accounted for 40% of the isolates and, similar to 2016 included ST1, ST5, ST8, ST30, ST45 (Fig. 8). While ST1 continued to predominate, a significant increase was also detected among isolates associated with ST30. *spa* type t127 continued to be the most frequently occurring. This strain has been associated with a prolonged outbreak throughout 2014 and increased awareness of the phenotypic characteristics of the strain in other hospitals has led to an increase in the number of isolates receipted in the NMRSARL.

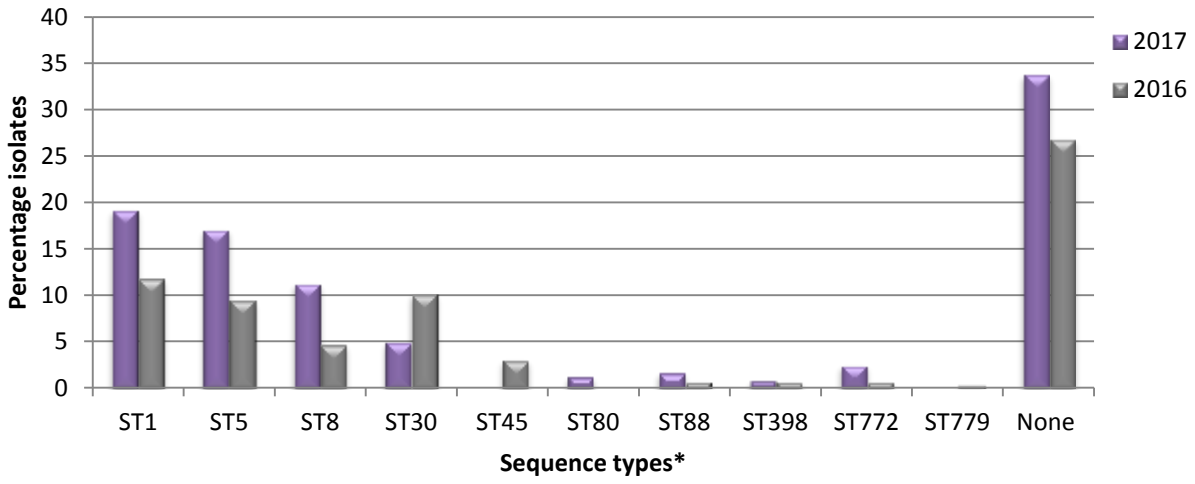


Figure 8. Most frequently recognised MLST among MRSA isolates investigated by *spa* typing during 2017.

***Sequence type inferred from data available on the Ridom *spa* typing database**

EMERGING STRAINS OF MRSA IN IRELAND

Previously MRSA was clearly defined as healthcare associated (HCA-) , community acquired (CA-) or livestock associated (LA-) however the lines of separation are becoming increasingly blurred with the importation of CA-MRSA strains into healthcare facilities and the zoonotic spread of LA-MRSA to humans. Furthermore, in Ireland over time, a strain displacement has occurred resulting in the ST22-MRSA-IV predominating in healthcare facilities. This displacement has also been reported in other countries where, once community associated strains have now become the predominant hospital associated strains (USA 300 in America and ST772 in India). Many of the strains recognised in Ireland have been reported elsewhere and very often, these strains exhibit greater resistance and harbour more virulence genes than the ST22 strains and so close monitoring is required in order to control the spread of these strains in the hospital setting.

mecC mediated LA-MRSA

Since 2011, *mecC* MRSA has been reported in MRSA recovered from humans, livestock, wild animals and companion pets throughout Europe. While *mecC* has not been identified in Ireland since 2010, in 2017 there were four isolates harbouring *mecC* submitted to the laboratory. Three isolates were recovered from humans in the South, South East and West of the country while the remaining isolate was recovered from a companion pet in Dublin. Elsewhere *mecC* has frequently been associated with MRSA recovered from animal sources; however this isolate is the only case of a *mecC* isolate recovered from an animal in Ireland.

CC398-MRSA

First reported among pigs in the Netherlands in the early 2000s CC398 LA-MRSA has since been reported among a range of livestock and horses, as well as in humans in several European countries along with America and Australia. In addition some reports suggest that CC398 MRSA accounts for up to 25% of all community-associated MRSA in some parts of Europe. Although first detected in 2012 in Ireland, CC398-MRSA continues to be relatively rare with only two incidences detected in 2017. Furthermore, although traditionally associated with livestock, one of the isolates harboured the PVL genes suggesting human adaptation of the strain.

Whole genome sequencing to investigate outbreaks caused by CA-MRSA lineages

Molecular typing techniques have been highly useful in the monitoring the spread of MRSA strains in healthcare facilities. However often such strains cannot be sufficiently differentiated by means of traditional DNA based methods. More recently, improvements in both the time-to-results and the affordability of whole-genome sequencing (WGS) have allowed the NMRSARL to explore this technology in the typing of strains causing outbreaks in Irish healthcare facilities.

During 2017, the NMRSARL involved in the publication of a study of ST1-t127-MRSA-IV isolates recovered from community and healthcare sources between 2013 and 2016 in order to investigate the isolate relationships and the extent of their spread. These community associated MRSA strains were selected for study due to their association with a prolonged hospital outbreak. Furthermore, this strain has increased in prevalence in recent years from <1% in 2010 to 11% of all those investigated by *spa* typing in 2016 (5).

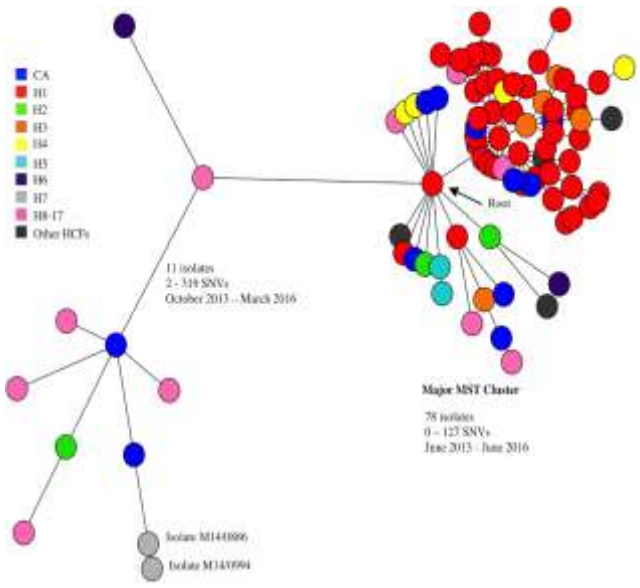


Figure 9. The pairwise single nucleotide variation (SNV) range between isolates inside and outside of the major minimum spanning tree (MST) cluster and their recovery time frame are indicated (5).

Core-genome MLST and Single nucleotide variant (SNV) analyses revealed the recent emergence and extensive spread of two closely related strains and multiple sporadic strains of ST1-MRSA-IV-t127/t922. Within the isolates recovered from an outbreak in a large Dublin hospital, isolates of this clone were predominantly multi drug resistant and frequently high-level mupirocin resistant (Hi-MupR), the latter of which can negatively affect efforts to eradicate carriage in colonized individuals.

During 2017 the NMRSARL was also involved in a study of the emergence of CC88-MRSA-IV in Ireland, a strain which, although present for several years has been associated with two outbreaks several years apart in a single facility. In this study isolates recovered from Ireland were compared with a global collection of isolates of the same strain.

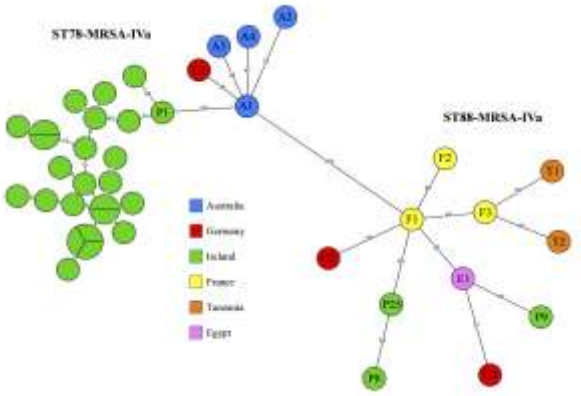


Figure 10. MST based on cg-MLST profiles of Irish and international CC88-MRSA isolates recovered between 2001 and 2017. The counties in which the isolates were recovered are indicated by the colour legend. Isolates were identified as either ST78-MRSA-IVa or ST88-MRSA-IVa. Branch labels represent allelic distances (6).

A cgMLST-based comparison of all isolates revealed that the outbreak strain was most likely imported from Australia, where it is among the prevalent MRSA clones. Furthermore the study also identified a second CC88-MRSA clone present in Irish hospitals, ST88-MRSA-IVa, which was likely imported from Africa, where it is predominant, and/or a country with a large population of African ethnic origin(6).

EDUCATION

The NMRSARL plays a prominent role in the education of laboratory staff and clinical staff.

In particular, NMRSARL staff gave lectures to undergraduate and post graduate students in the Dept. of Clinical Microbiology, TCD and the Dublin Institute of Technology. Scientific staff shared techniques used in the NMRSARL with staff from other hospital laboratories, research facilities, undergraduate students, transition year students and provided expert knowledge to students of other laboratories completing higher degrees.

CONTINUOUS PROFESSIONAL DEVELOPMENT

The level of expertise and knowledge among staff of NMRSARL is maintained through the participation of staff at both national and international meetings, workshops and conferences. Throughout the year all staff continued their professional development through attending some of the following meetings;

- Journal clubs
- Focus on Infection
- Antimicrobial Resistance
- Microbiology Advisory Body

NMRSARL staff also ensured mandatory training requirements were met in areas such as;

- Risk Management
- Chemical safety awareness
- Manual Handling & Fire safety
- Quality Management
- Hand Hygiene
- Transport of patient specimens

One member of staff completed a research PhD on the Characterisation of sporadically occurring MRSA in Ireland through the Dublin Dental University Hospital, TCD.

RESEARCH HIGHLIGHTS

NMRSARL continues to participate in several collaborations with both local and international groups in order to enhance the research in the field of *S. aureus* in Ireland.

Whole genome sequencing

- Evaluation of core genome MLST typing methods for the routine use of WGS in outbreak situations

Emerging MRSA strains

- Monitoring of the characteristics of novel and potentially emerging MRSA clones e.g. ST772-MRSA-V, and ST1-MRSA-IV which carry multiple resistance and virulence genes and have been involved in outbreaks in healthcare facilities

CA-MRSA

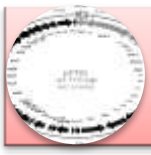
- Characterisation of the genotypes, virulence and antimicrobial resistance genes of *pvl*-positive MRSA in Ireland and MRSA in closed communities

LA-MRSA

- Investigation of MRSA from animal populations for the presence of *mecC* in order to determine if isolates harbouring this gene are a significant problem among LA-MRSA isolates Ireland or if the zoonotic spread of these MRSA strains are contributing to the burden of MRSA among humans

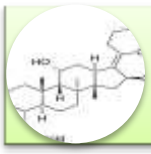
MSSA

- Investigating the genotypes, virulence and antimicrobial resistance potential of MSSA isolates associated with BSI) and MRSA from BSIs in order to investigate why MSSA BSIs are increasing in Ireland while MRSA BSIs are decreasing



Mupirocin resitance

- Investigation of the genotypes of Hi-MupR MRSA isolates and in-depth analysis of Hi-MupR-conferring plasmids



Fusidic acid resistance

- Investigation of the genetic mechanism of fusidic acid resistance in MRSA in Ireland

Linezolid resistance

- Investigation of linezolid resistance among MRSA, CoNS and VRE and particularly resistance encoded for by the *cfr* and *optrA* genes

PUBLICATIONS

Below are abstracts resulting from these very successful collaborations which have been published or accepted for publication throughout the year.

The recent emergence in hospitals of multidrug-resistant community-associated sequence type 1 and spa type t127 methicillin-resistant Staphylococcus aureus investigated by whole-genome sequencing: Implications for screening.

Earls MR, Kinnevey PM, Brennan GI, Lazaris A, Skally M, O'Connell B, Humphreys H, Shore AC, Coleman DC.

PLoS One (2017) Apr 11; 12(4):e0175542.

Community-associated *spa* type t127/t922 methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence increased from 1%-7% in Ireland between 2010-2015. This study tracked the spread of 89 such isolates from June 2013-June 2016. These included 78 healthcare-associated and 11 community associated-MRSA isolates from a prolonged hospital outbreak (H1) (n = 46), 16 other hospitals (n = 28), four other healthcare facilities (n = 4) and community-associated sources (n = 11). Isolates underwent antimicrobial susceptibility testing, DNA microarray profiling and whole-genome sequencing. Minimum spanning trees were generated following core-genome multilocus sequence typing and pairwise single nucleotide variation (SNV) analysis was performed. All isolates were sequence type 1 MRSA staphylococcal cassette chromosome *mec* type IV (ST1-MRSA-IV) and 76/89 were multidrug-resistant. Fifty isolates, including 40/46 from H1, were high-level mupirocin-resistant, carrying a conjugative 39 kb iles2-encoding plasmid. Two closely related ST1-MRSA-IV strains (I and II) and multiple sporadic strains were identified. Strain I isolates (57/89), including 43/46 H1 and all high-level mupirocin-resistant isolates, exhibited ≤80 SNVs. Two strain I isolates from separate H1 healthcare workers differed from other H1/strain I isolates by 7-47 and 12-53 SNVs, respectively, indicating healthcare worker involvement in this outbreak. Strain II isolates (19/89), including the remaining H1 isolates, exhibited ≤127 SNVs. For each strain, the pairwise SNVs exhibited by healthcare-associated and community-associated isolates indicated recent transmission of ST1-MRSA-IV within and between multiple hospitals, healthcare facilities and communities in Ireland. Given the interchange between healthcare-associated and community-associated isolates in hospitals, the risk factors that inform screening for MRSA require revision.

Novel multiresistance *cfr*-plasmids in linezolid resistant methicillin resistant *Staphylococcus epidermidis* and vancomycin resistant *Enterococcus faecium* from a hospital outbreak: first report of co-location of *cfr* and *optrA* in VRE.

Lazaris A, Coleman DC, Kearns AM, Pichon B, Kinnevey PM, Earls MR, Boyle B, O'Connell B, Brennan GI, Shore AC,

J Antimicrob Chemother (2017) 72: 12; 3252-7

BACKGROUND:

Linezolid is often the drug of last resort to treat infections caused by Gram-positive cocci. Linezolid resistance can be mutational (23S rRNA or L-protein) or, less commonly, acquired [predominantly *cfr*, conferring resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A compounds ($PhLOPS_A$) or *optrA*, encoding oxazolidinone and phenicol resistance].

OBJECTIVES:

To investigate the clonality and genetic basis of linezolid resistance in 13 linezolid-resistant (LZDR) methicillin-resistant *Staphylococcus epidermidis* (MRSE) isolates recovered during a 2013/14 outbreak in an ICU in an Irish hospital and an LZDR vancomycin-resistant *Enterococcus faecium* (VRE) isolate from an LZDR-MRSE-positive patient.

METHODS:

All isolates underwent $PhLOPS_A$ susceptibility testing, 23S rRNA sequencing, DNA microarray profiling and WGS.

RESULTS:

All isolates exhibited the $PhLOPS_A$ phenotype. The VRE harboured *cfr* and *optrA* on a novel 73 kb plasmid (pEF12-0805) also encoding *erm*(A), *erm*(B), *lnu*(B), *lnu*(E), *aphA3* and *aadE*. One MRSE (M13/0451, from the same patient as the VRE) harboured cfr on a novel 8.5 kb plasmid (pSEM13-0451). The remaining 12 MRSE lacked *cfr* but exhibited linezolid resistance-associated mutations and were closely related to (1-52 SNPs) but distinct from M13/0451 (202-223 SNPs).

CONCLUSIONS:

Using WGS, novel and distinct *cfr* and *cfr/optrA* plasmids were identified in an MRSE and VRE isolate, respectively, as well as a *cfr*-negative LZDR-MRSE ICU outbreak and a distinct *cfr*-positive LZDR-MRSE from the same ICU. To our knowledge, this is the first report of *cfr* and *optrA* on a single VRE plasmid. Ongoing surveillance of linezolid resistance is essential to maintain its therapeutic efficacy.

RESOURCES

Staff

During 2017 the staff working in the NMRSARL were;

- Gráinne Brennan
- Tanya Fleming
- Paul Grier
- Fionnuala McGrath

Dr. Anna Shore continued in her role as a Lecturer in Applied and Translational Microbiology and, in this role continued her involvement in the development of applied research in MRSA between the School of Dental Science, Trinity College and NMRSARL.

The role of Director was discharged in an honorary capacity by Dr. Brian O'Connell, Consultant Microbiologist, SJH. Professor Hilary Humphreys of the Royal College of Surgeons in Ireland and Beaumont Hospital continued in his role of Honorary Consultant to provide an external perspective to the activities and services provided by NMRSARL.

Facilities

NMRSARL consists of three main laboratory areas, a Phenotyping Laboratory, a Genotyping Laboratory and a PCR Laboratory. The provision of a suitable computer system is a major requirement, both for monitoring isolates received and for detailed analytical work.

The NMRSARL is included in the National MedLis project and is working the relevant parties to ensure the requirements of the NMRSARL are met.

Finance

The budget allocated to the NMRSARL for the year to cover both pay and non-pay elements amounted to € 301,238.

Administration

The laboratory is located in St. James's Hospital and is administered within the Laboratory Medicine (LabMed) Directorate.

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