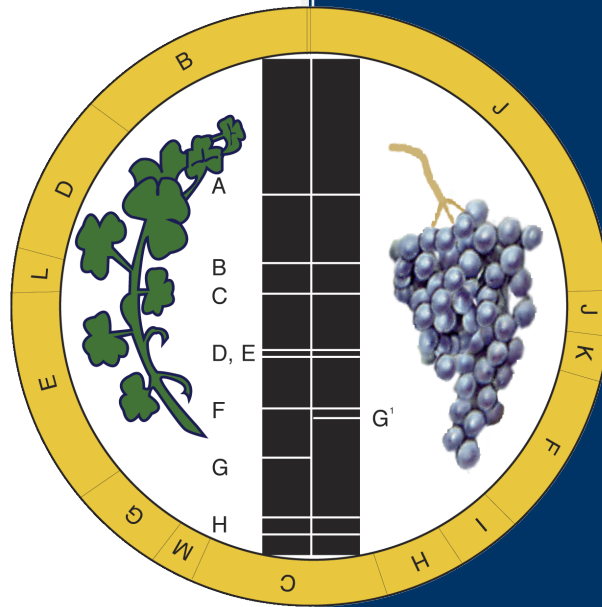


ANNUAL REPORT 2021



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 2021. Despite changes to working practices arising due to Covid, throughout 2021, the laboratory continued to deliver on its role in assisting medical professionals is the control of MRSA in hospitals and the community in Ireland.

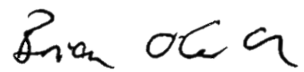
In addition to the routine work of the laboratory which includes molecular typing for epidemiological investigation and the detection of important virulence factors, service developments and projects during the year included:

- 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 and whole genome sequencing;
- 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 continued several validations to lead to service improvement including that of the MiSeq which, will enable the laboratory to provide users of the laboratory with assistance when investigating outbreaks caused by MRSA.

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

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SUMMARY

Public health impact	<ul style="list-style-type: none">•The surveillance and identification of potential outbreaks of MRSA and MSSA•The laboratory also monitors the incidence of <i>pvl</i> carrying strains of <i>S. aureus</i> and the strains associated with healthcare infections
New service developments	<ul style="list-style-type: none">•Phenotypic and genotypic methods remain under constant review to take advantage of any newly developed methodologies;•Investigation of transferrable resistance genes encoding linezolid resistance in enterococci and Staphylococci
Activity	<ul style="list-style-type: none">•During 2021, the EARS-Net project accounted for 9.8% of the overall workload of the NMRSARL while MSSA isolates and non <i>S. aureus</i> isolates accounted for 55.3%•Further increase in the uptake of newer services including whole genome sequencing and investigation of linezolid resistance among Enterococci and CoNS
Research and development	<ul style="list-style-type: none">•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	<ul style="list-style-type: none">•The laboratory continues to offer training to biomedical science students and postgraduate students in Trinity College Dublin and Dublin Institute of Technology
Future developments	<ul style="list-style-type: none">•The use of whole genome sequencing for outbreak investigation and characterisation of <i>S. aureus</i> and Enterococci isolates.

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 methicillin 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 year) to NMRSARL where they are investigated for resistance to oxacillin, vancomycin and teicoplanin using standard gradient MIC strip and 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	90	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	634	Surveillance, recognition, investigation and management of PVL <i>S. aureus</i> in Ireland
MRSA & MSSA	Surveillance analysis and identification of trends	679	Typing and susceptibility testing of MRSA and MSSA isolates submitted throughout the year.
MRSA and MSSA	Surveillance	37	Outbreak/cluster investigations (n=68) throughout Ireland
MRSA and MSSA	Confirmation of resistance against various antibiotic agents	800	Confirmation of resistance against glycopeptides, β -lactams, daptomycin and newer agents.
VRE and CoNS	Confirmation of linezolid and other resistance	159	Characterisation of resistance mechanism associated with increased resistance in VRE and CoNS

REFERENCE LABORATORY WORK

As expected MRSA isolates accounted for the largest proportion (44.7%) of the laboratory workload during 2021. Isolates recovered from bloodstream infections that were investigated under the European Antimicrobial Resistance Surveillance Network (EARS-Net) accounted for 9.8% of the overall workload and MRSA recovered from other sites being 34.9%. As in previous years the number of requests to investigate susceptible *S. aureus* and other Gram positive organisms have also increased now representing 38.2% and 17.1% respectively (Fig. 1).

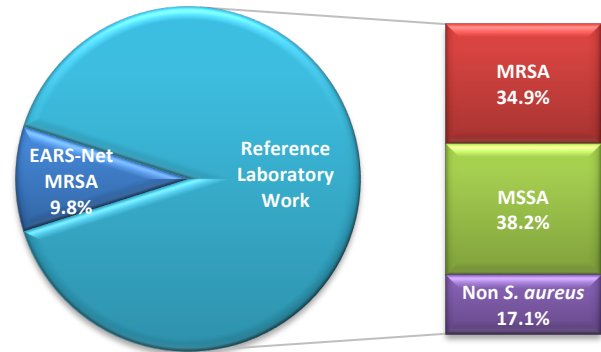


Fig 1 Workload of the NMRSARL during 2021

In recent years an increase in requests for investigations of MSSA isolates has led to a change in the services of the laboratory and 2021 saw a further increase in the uptake of newer services including the investigation of linezolid resistance among Enterococci and CoNS.

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 staphylococci submitted using disk diffusion whilst all enterococci undergo MIC determination using broth microdilution. Further molecular investigation is also performed on over half of these isolates including investigation for resistance and virulence gene (n=681) or *spa* typing (n=416). 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.

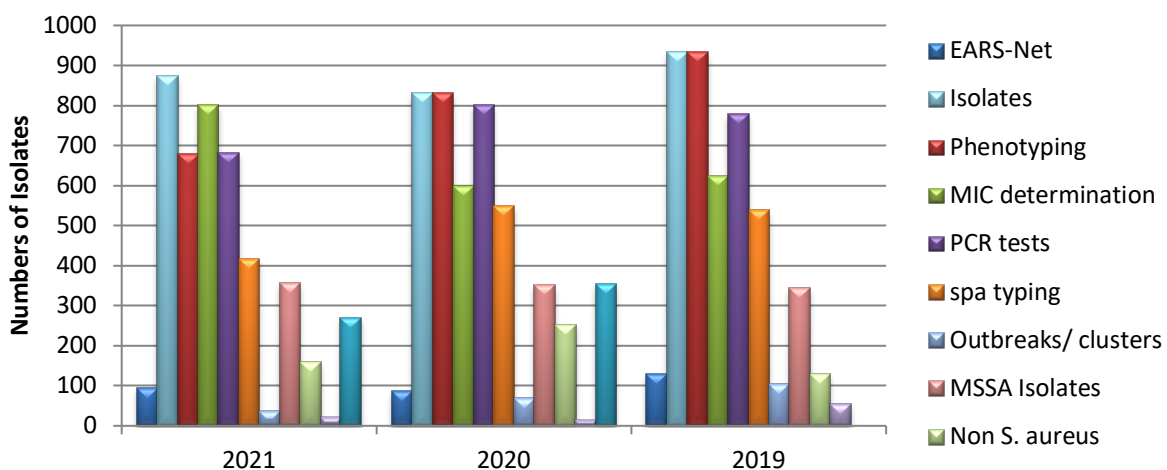


Fig 2 Distribution of workload throughout 2021

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. However, the emerging community associated strains carry multiple virulence and resistance genes including those associated with aminoglycoside and tetracycline resistance.

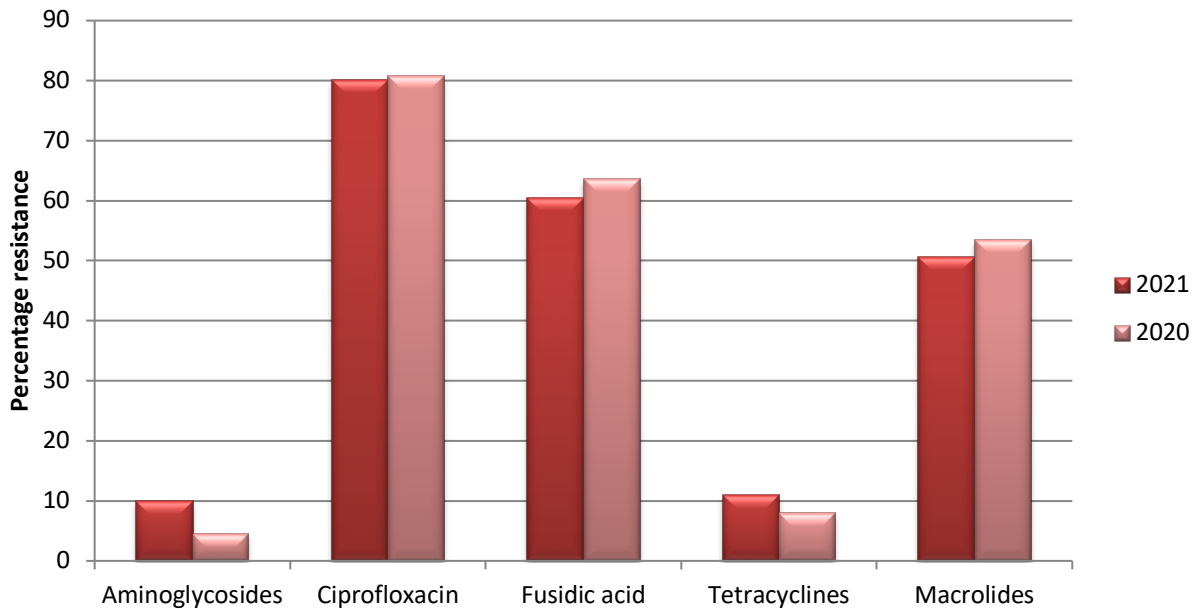


Fig 3 Resistance rates among EARS-Net isolates recovered in 2021

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. 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 57% of isolates exhibiting multi-antibiotic resistance, that is, resistance to three or more different classes of antibiotics and to aminoglycosides, mupirocin, and tetracycline.

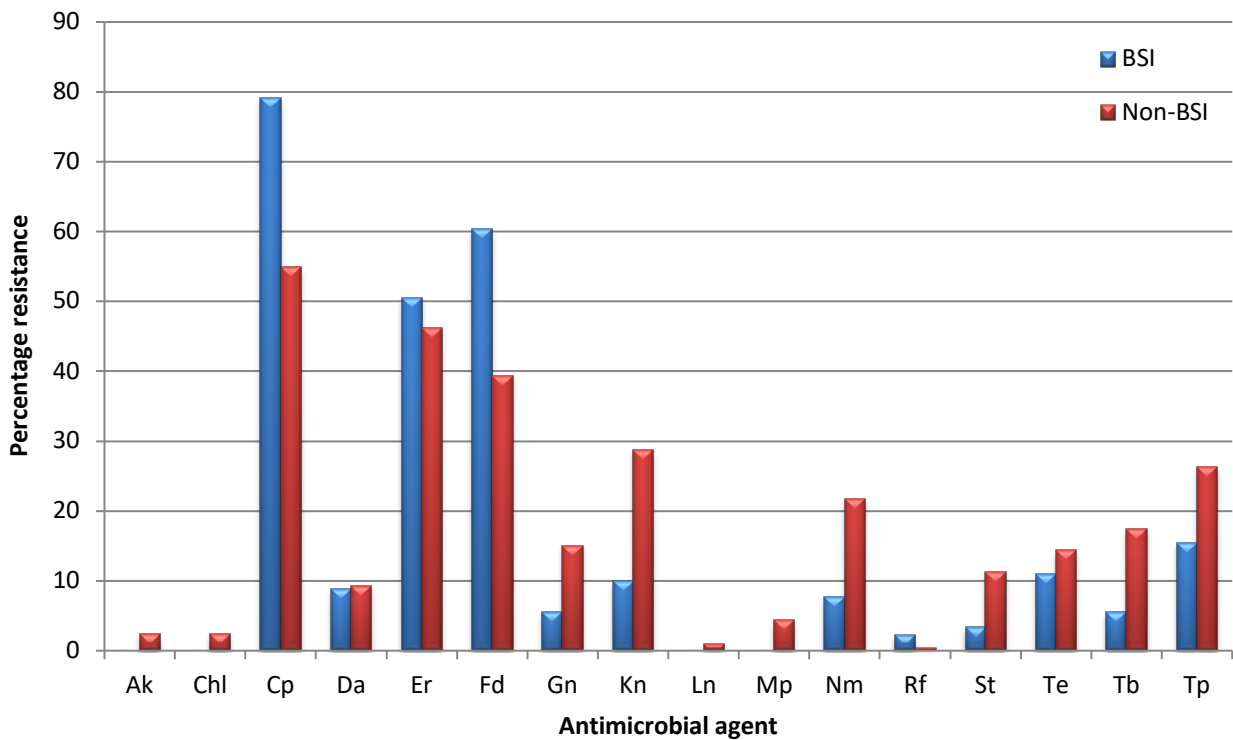


Fig 4 The percentage of blood stream MRSA isolates exhibiting resistance to each antimicrobial agent in comparison to those recovered from non-blood stream infections.

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

Antimicrobial resistance to newer agents

Surveillance studies provide important information in the identification of trends in the development of antimicrobial resistance. Monitoring of newer agents for treatment of MRSA infection is important as resistance detection is rare and difficult with not all laboratories routinely testing against these agents.

Whilst the NMRSARL has monitored susceptibility to several agents using gradient MIC strips for several years, in 2019 the laboratory introduced broth microdilution investigation for linezolid, daptomycin, ceftaroline, dalbavancin, vancomycin, tigecycline and telavancin. Broth microdilution is highly accurate method for MIC determination and is often considered the gold standard of susceptibility testing.

The MIC was determined by broth microdilution on all isolates submitted as part of the EARS-Net project. The distribution of the MICs observed for each agent is shown below and is compared to the MIC observed for isolates from 2020.

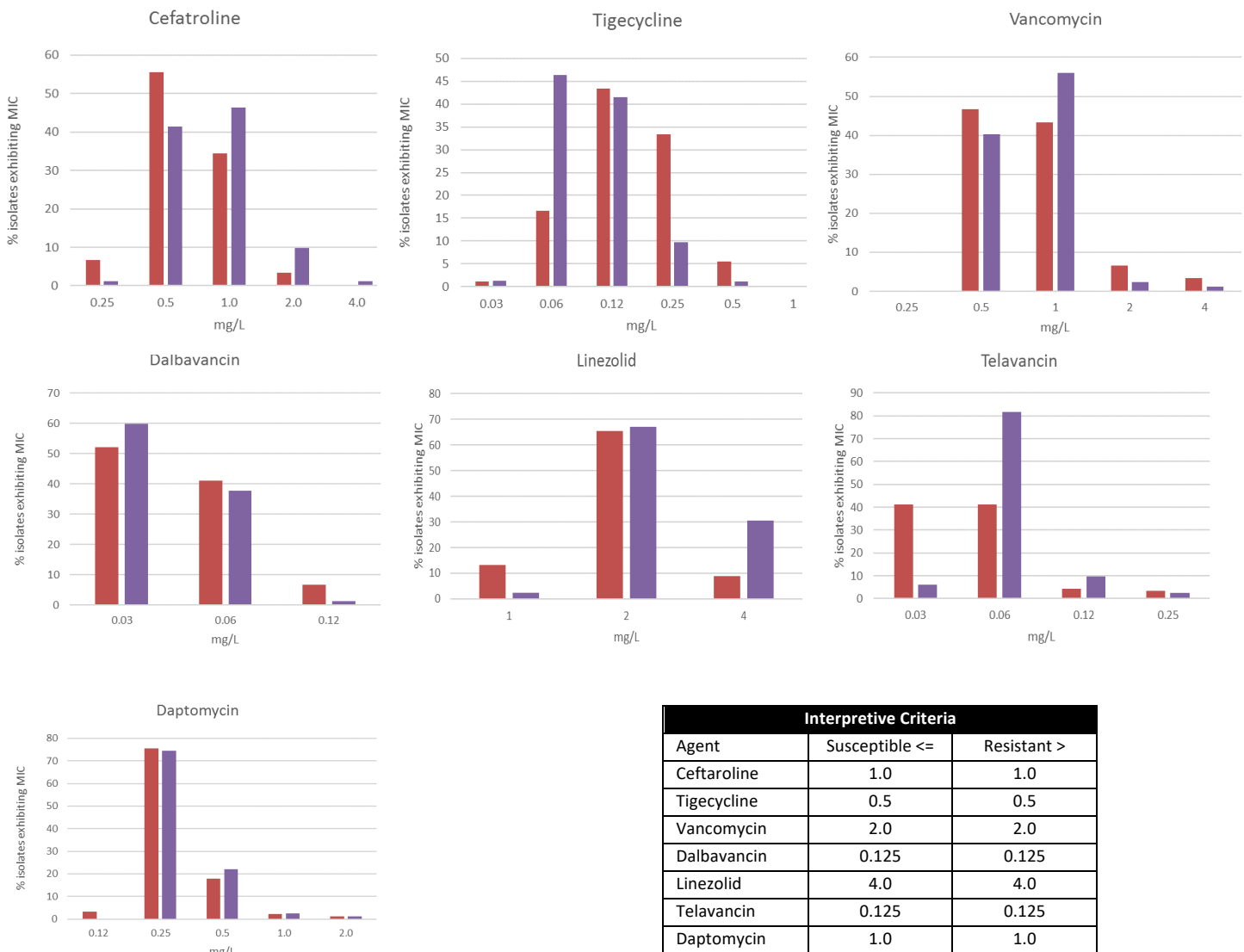


Fig 5 The minimum inhibitory concentration (MIC) of MRSA isolates recovered from blood stream infections in 2021 compared to those recovered in 2020

Linezolid resistance in Staphylococci and Enterococci

In 2019 Ireland had one of the highest proportions of vancomycin resistant *Enterococci faecium* (VRE_{fm}) in Europe. In addition, in recent years an increase in resistance to linezolid has also been reported. Since 2016 the NMRSARL has investigated linezolid resistance in Enterococci and Staphylococci for the presence of *cfr* and *optrA* (2). In 2018 this was expanded to include the gene *poxtA* with the mutation G2576T included to the list of resistance determinants investigated in 2021 (3).

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 while, along with these *poxtA* also encodes resistance to tetracyclines.

Work carried out in the NMRSARL has found isolates recovered in Ireland can carry multiple resistance mechanisms. In 2017 an *E. faecium* isolate was found to harbour both *cfr* and *optrA* while in 2018 an isolate was found to be positive for *optrA* and *poxtA*.

During 2021 there were 94 isolates investigated for these resistance genes. Among isolates investigated 64.2% (60/94) exhibited phenotypic resistance and of these 26.6% (17/64) harboured *optrA*, 4.7% (3/64) were found to harbour *poxtA* and one isolate harboured both *optrA* and *poxtA*. Detection of mutational resistance associated with 23S rRNA was introduced during 2021 and of those investigated for the G2576T mutation 20 isolates were found to be positive. There were no isolates positive for the *cfr* gene.

EPIDEMIOLOGICAL TYPING OF MRSA IN IRELAND

For several years, the NMRSARL has used phenotypic and molecular epidemiological typing techniques. Molecular techniques include *spa* typing, which has been shown to have good concordance and congruence with MLST and enable the NMRSARL to report inferred MLST data based on the *spa* type. Since 2019 however, all isolates submitted to the NMRSARL for investigation under the EARS-Net project also undergo whole genome sequencing.

Whole genome sequencing (WGS) found that, like previous years, ST22-MRSA-IV continues to be the predominant strain circulating in healthcare settings this predominance is decreasing and an increase in the diversity of strains continues to be observed. This strain is also known as UK-EMRSA-15, Barnim Epidemic Strain, Spanish PFGE type E13, or Canadian MRSA-8 and has been increasing in Ireland since the late 2000s (4).

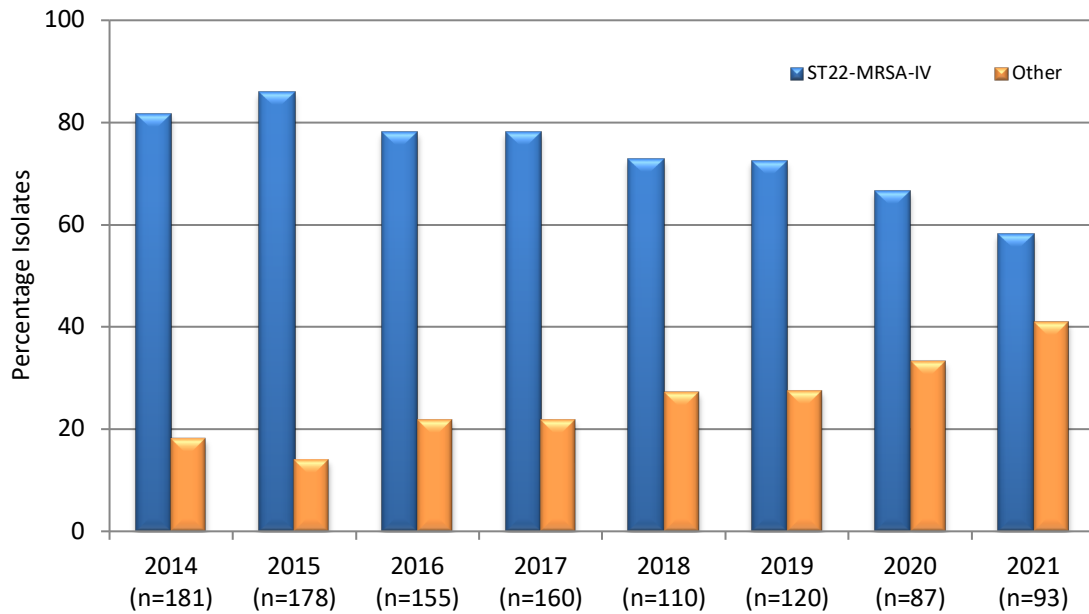


Fig 6 Epidemiological types of MRSA strains recovered from blood stream infections. 2012-2020. 2012-2018 MLST types inferred using *spa* typing and antibiogram resistogram (AR) typing.

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 to control the spread of these strains in the hospital setting.

Unlike in previous years when all non-ST22-MRSA-IV were classed as “other”, WGS has also enabled us to determine the genetic profile of these strains. These included ST1, ST5, ST8, ST30, ST45, ST59, ST398 and ST772. Information about these strains is limited due to the infrequency in which they are reported however in Ireland:

- ST1, ST5 and ST30 are frequently associated with CA-MRSA;
- ST398 was previously considered livestock associated MRSA however now more is frequently associated with CA-MRSA (*pvl*-positive) among people with epidemiological links to Southeast Asia;
- ST772 is a *pvl*-MRSA-V strain that has been associated with the Bengal Bay region and has caused several outbreaks in Irish Healthcare settings.

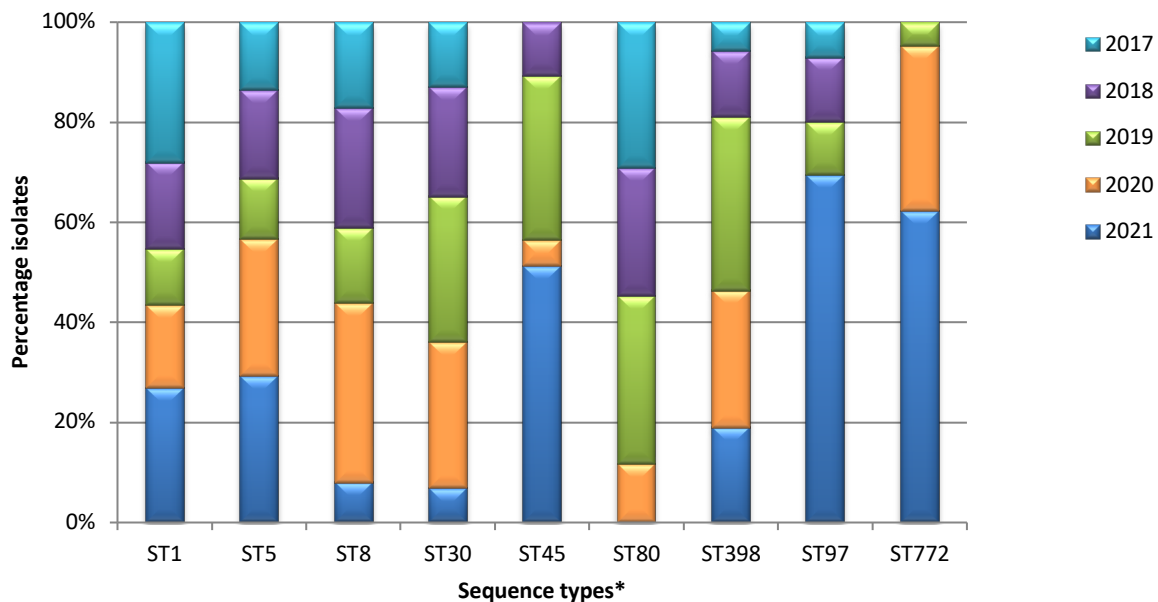


Fig 7 Epidemiological types of MRSA strains recovered from blood stream infections. 2012-2020. 2012-2018 MLST types inferred using *spa* typing and antibiogram resistogram (AR) typing. MLST assigned to isolates investigated in 2019-2020 following whole genome sequencing analysis. The total number of isolates investigated each year is shown in parentheses. Also shown is the percentage of isolates assigned to each sequence type each year.

ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND

Like Europe, ST22-MRSA-IV is the pandemic clone in Ireland and, in 2021, was associated with 66.6% of MRSA causing blood stream infections. This strain is also known as UK-EMRSA-15, Barnim Epidemic Strain, Spanish PFGE type E13 or Canadian MRSA-8 (4).

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 and has also been recovered from companion animals such as horses, cats and dogs (4).

Due to the low discriminatory power of current bacterial epidemiological typing techniques such as *spa* typing when differentiating ST22-MRSA-IV in Irish hospitals, the NMRSARL utilized whole genome sequencing technology to provide detailed analysis of the ST22-MRSA-IV isolates (n=53) recovered from blood cultures during 2021.

Core genome multi locus sequence typing (cg-MLST) is an allele-based approach used to interpret whole genome sequencing data. cgMLST involves the comparison of 1,861 core genes and allows clustering of closely related isolates. For MRSA, whilst there are no

definitive cgMLST thresholds for assigning isolate relatedness, a difference of ≤ 24 alleles may be used as an approximate clonality guideline. Among the 2021 EARS-Net collection, there were seven occasions where isolates had fewer than 24 differences several of which involved isolates from different hospitals.

A maximum-likelihood phylogenetic tree was reconstructed to illustrate the ancestral relationships between the ST22-MRSA-IV isolates based on a core genome alignment. The tree was annotated with the distribution of all identified resistance genes (Fig 7).

Common resistance patterns exhibited by the ST22-MRSA-IV strain include resistance to fusidic acid, ciprofloxacin, and erythromycin. Associated resistance genes detected included *blaZ* (β -lactamase), *erm(C)/lnu(A)* (macrolides) and *ant(4)/aph(2)* (aminoglycosides) (Fig 6). Separately, other mutational resistance determinants recognised included *fusA* (fucidic acid) and *gyrA* (ciprofloxacin). Variable virulence markers detected ST22-MRSA-IV are *sec* and *sel* as well as the IEC genes encoded by lysogenic β -haemolysin-converting phages (*sak*, *chp*, *scn*).

A separate report has been sent to each hospital detailing the results of isolates submitted from their laboratory.

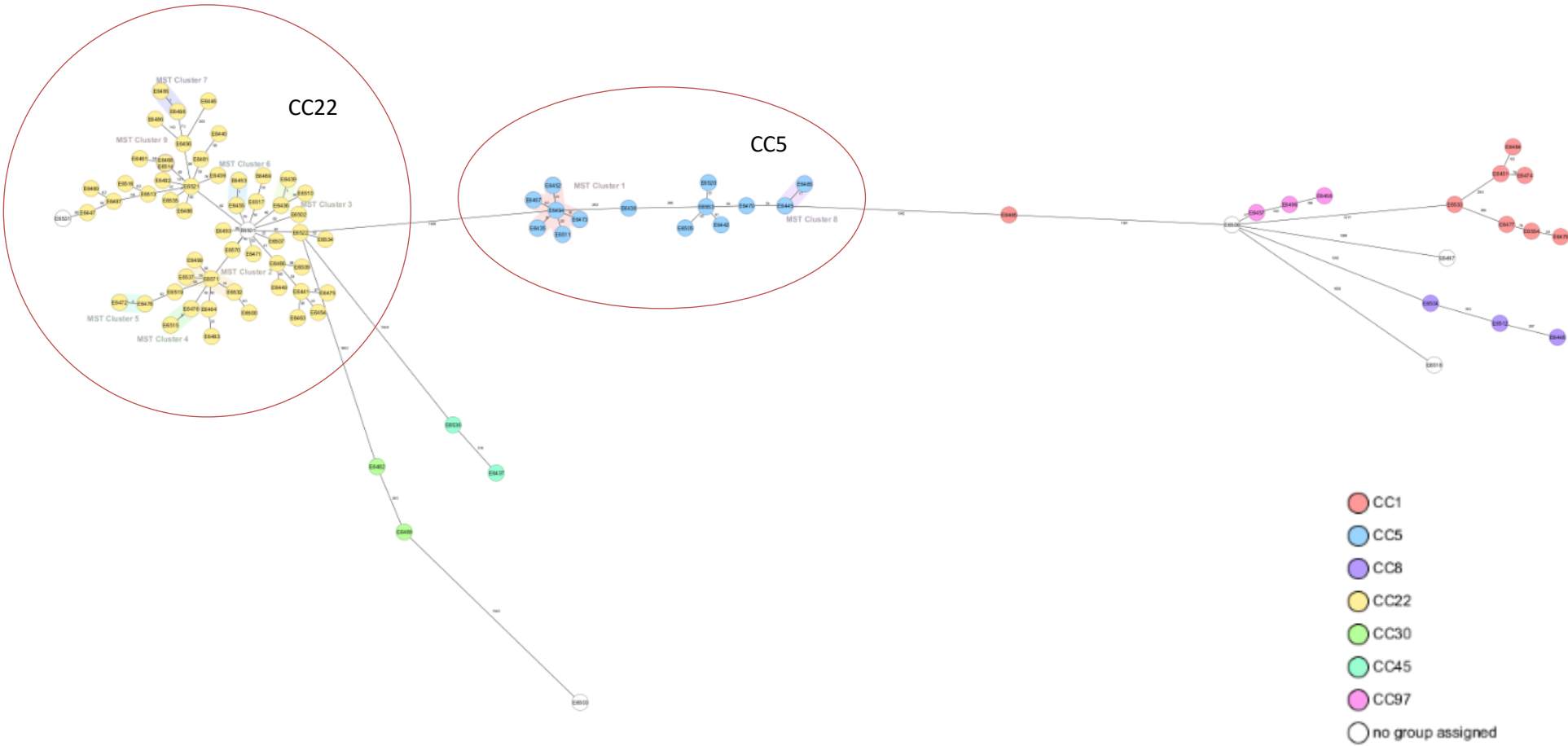


Fig 9: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all MRSA isolates recovered from blood stream infections during 2021. In each MST, MRSA isolates assigned to the same sequence type (ST) are indicated by separate colours. Closely related clusters of isolates (C1-8) (≤ 24 cgMLST allelic differences) are outlined with grey shading. The numbers on each branch indicate the numbers of cgMLST allelic differences detected between neighbouring isolates. All clusters of closely related isolates were identified among those isolates assigned to CC22 and CC5.

PVL positive *S. aureus*

Throughout 2021 the detection of PVL continued to be the most frequently requested test. The PVL toxin is a cytotoxicigenic 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 2021, 573 *S. aureus* isolates (non-BSI) were investigated for carriage of the *lukS-PV* and *lukF-PV* genes encoding for PVL. The isolates investigated included 257 MRSA and 316 MSSA.

Among the MRSA isolates 15.6% (40/257) were found to be positive while 10.1% (32/316) of MSSA isolates were also positive.

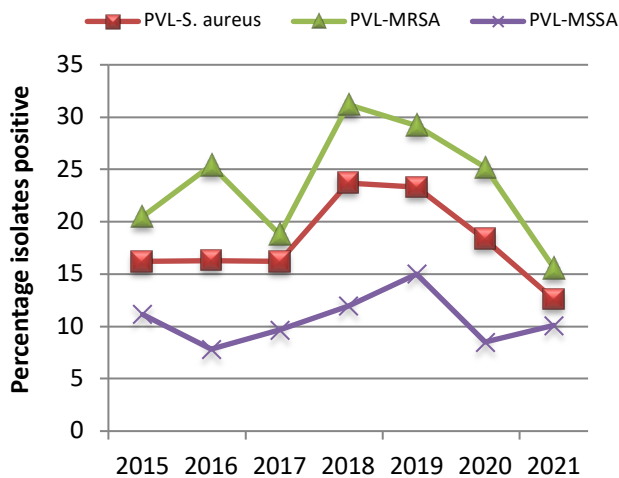


Fig 12: Frequency of PVL *S. aureus*

The change in the number of PVL-positive MRSA in 2020 in comparison to previous years was primarily due to several outbreaks and clusters

in healthcare settings identified during previous years.

As in previous years, the distribution of epidemiological types among PVL+ *S. aureus* is limited with less diversity seen among the MRSA isolates. In 2021, 62.5% of the isolates were limited to only eight sequence types with a further the 22% not assigned to any MLST by *spa* typing (Fig 10).

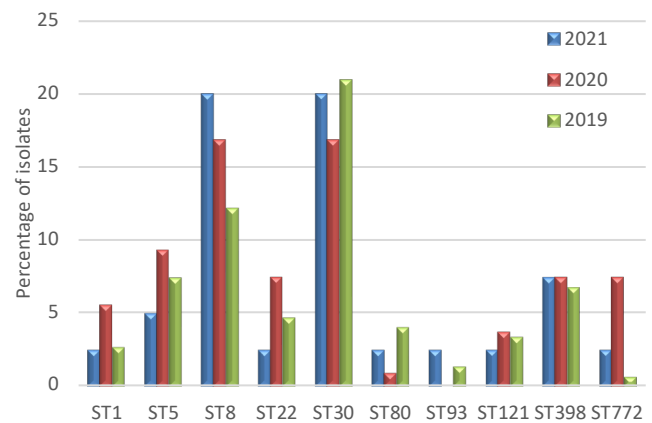


Fig 13 Distribution of sequence types among PVL-S. aureus isolates recovered in 2020.

Both MRSA and MSSA were found to be associated with only five STs (ST1, ST5, ST8, ST30 and ST398). Among the most frequently recognised strains ST8 and ST30 are globally disseminated while ST398 are often associated with severe skin and soft tissue infections often with epidemiological links to Southeast Asia.

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. Whilst EARS-Net undergo WGS, resource constraints limit the number of additional isolates which undergo sequencing. However, a large proportion of isolates undergo *spa* typing on an annual basis allowing easier comparison of MRSA recovered in Ireland with those recovered elsewhere throughout the world.

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 (5).

Using the inferred MLST data available from the *spa* typing online database the most frequently recognised MLST types accounted for over 35% of the isolates and, like previous years, included ST1, ST5, ST8 and ST30 (Fig 1).

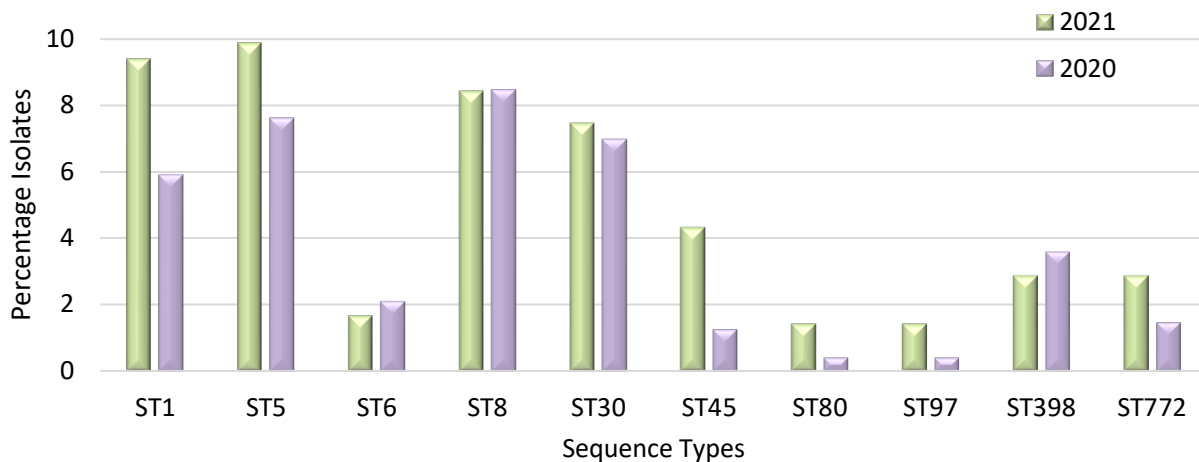


Fig 14 Most frequently recognised MLST among MRSA isolates investigated by *spa* typing during 2021

*Sequence type inferred from data available on the Ridom *spa* typing database. Inferred MLST were not available for 32% of *spa* types (n=131) recognised.

WHOLE GENOME SEQUENCING TO INVESTIGATE CA-MRSA LINEAGES RECOVERED IN IRISH HEALTHCARE SETTINGS

In recent years the NMRSARL has been involved in several studies investigating the emergence of different lineages of MRSA in Ireland especially those which have been associated with outbreaks in healthcare facilities (6, 7).

These have included:

- PVL positive t002-CC5-MRSA-IV (n=9) causing a prolonged outbreak in a neonatal ICU;
- A cluster of *Staphylococcus argenteus* recovered in a paediatric unit.
- PVL positive t008-CC8-MRSA-IV causing an outbreak in a neonatal ICU (n=7) and at the same time a cluster of isolates recovered from patients in a nearby hospital (n=6).
- PVL positive t127-ST1-MRSA-V recovered from a previously MRSA negative patient during a prolonged stay within a hospital.

Since 2019, many of these strains associated with outbreaks have undergone whole genome sequencing. Below shows an MST of all strains associated with CA-MRSA which have undergone whole genome sequencing in the NMRSARL (Fig 12). 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.

As shown in Fig 12, over 30 clusters have been recognised to date among CA-MRSA lineages, which included isolates spanning several years and were in both community and healthcare settings. The largest clusters were caused by isolates from ST1, ST5, ST6 and ST772.

- t304-ST6-MRSA-IV: This strain has been recovered from a number of hospitals and increased in prevalence in recent years now accounting for 1.5-2% of non ST22 lineages and has been associated with two prolonged outbreaks. These strains are non-multidrug resistant, have been associated with outbreak in healthcare facilities in other countries and do not usually lead to serious infections. It has also been suggested that t304-ST6 has been imported into Norway through immigration from the Middle East however there is limited epidemiological information available on isolates recovered in Ireland to determine similar links;
- PVL-t002-ST5-MRSA-IV: This strain has been associated with outbreak in two different healthcare facilities. It has recently been described as a Sri Lankan clone and has also been reported in Australia and the UK;
- Other larger outbreaks were caused by t127 (ST1), t1802 (ST5) and t1597 (ST72), all of which have been previously reported in other countries.

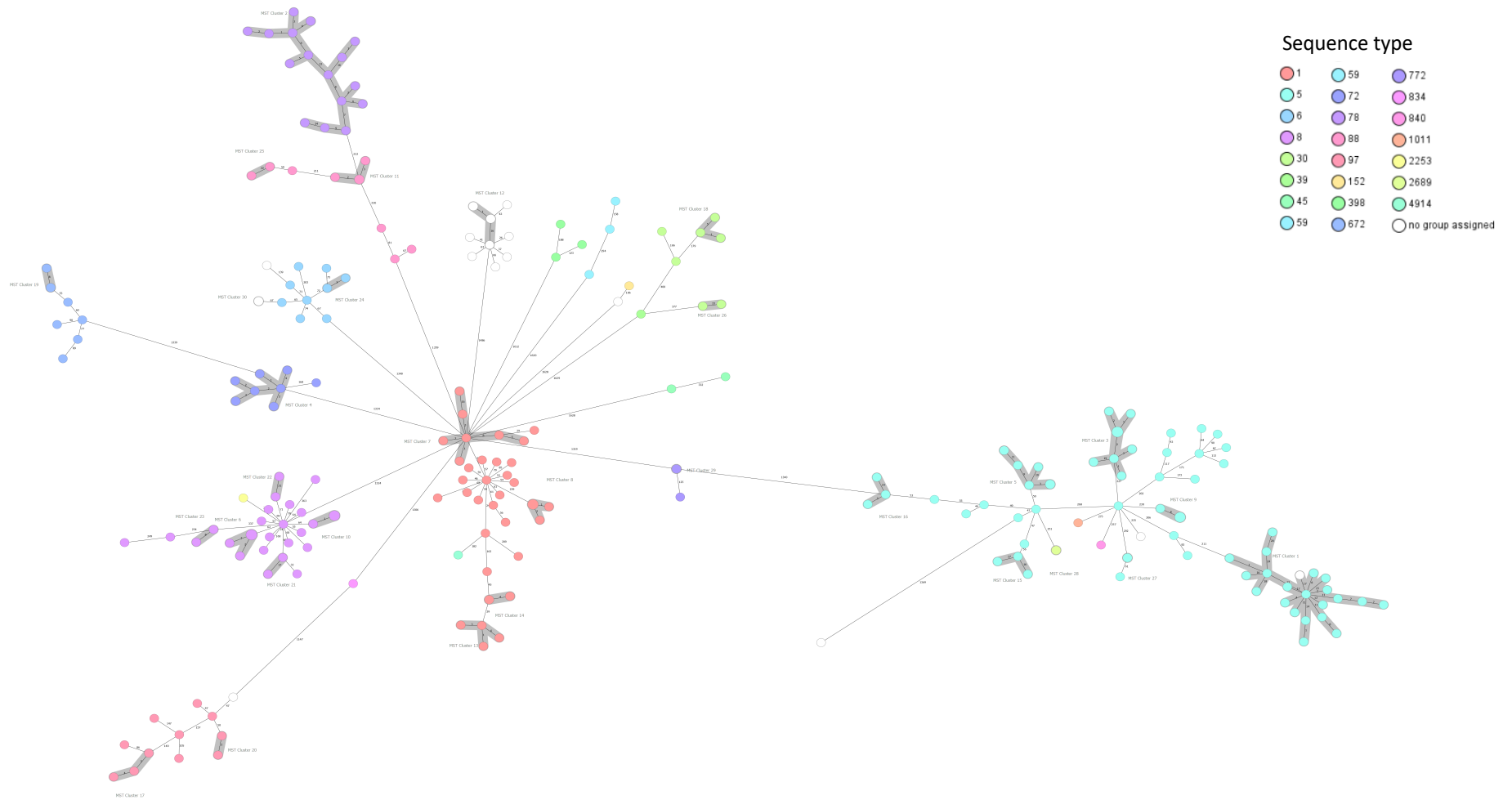


Fig 15: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all MRSA isolates associated with CA-MRSA lineages investigated by whole genome sequencing. In each MST, MRSA isolates assigned to the same sequence type (ST) are indicated by separate colours. Closely related clusters of isolates (≤ 24 cgMLST allelic differences) are outlined with grey shadowing. The numbers on each branch indicate the numbers of cgMLST allelic differences detected between neighbouring isolates.

EDUCATION

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

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.

The laboratory also assisted in several post graduate students undertaking projects including epidemiological typing of MRSA recovered from maternity hospitals, investigation of CoNS and MSSA from diabetic patients and characterisation of CA-MRSA.

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;

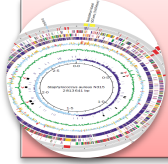
- Journal clubs
- Focus on Infection
- Antimicrobial Resistance
- Microbiology Advisory Body

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

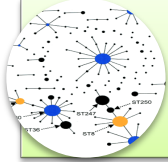
RESEARCH HIGHLIGHTS

NMRSARL continues to participate in several collaborations with both local and international groups to enhance 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



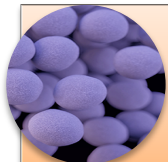
CA-MRSA

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



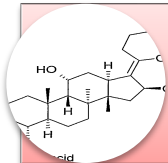
LA-MRSA

- 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 resistance



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.

Broderick D, Brennan GI, Drew RJ, O'Connell B. Epidemiological typing of methicillin resistant *Staphylococcus aureus* recovered from patients attending a maternity hospital in Ireland 2014-2019. *Infect Prev Pract.* 2021 Jan 24;3(1):100124.

Summary

It has previously been reported that maternity hospitals have lower levels of MRSA compared to other healthcare facilities, due to the patient population - mostly healthy patients with limited healthcare contact. In this epidemiological study, all MRSA isolates recovered from patients attending a maternity hospital from 2014 – 2019 were investigated.

171 isolates from adults (n=120) and babies (n=51) from diagnostic and screening investigations were submitted to the National MRSA Reference Laboratory (NMRSARL). Investigations included: spa typing, antimicrobial susceptibility testing, detection of the *mecA/mecC* genes and *lukS-PV* and *lukF-PV*. All were susceptible to glycopeptides, linezolid, rifampicin and mupirocin, while 29 of 171 (17%) were resistant to β -lactam agents only. Thirteen isolates (8%) were resistant to two classes of antibiotic; one resistant to three. All isolates harboured *mecA* and 33 of 171 (19%) harboured *PV-lukF/S*.

Among the collection, 21 multilocus sequence types (ST) were inferred from 63 spa types. EARS-NET data shows that ST22-MRSA-IV accounts for approximately 75% of MRSA recovered in Irish hospitals. Here, it accounted for only 25.7%. MLST types associated with community acquired MRSA accounted for the remaining 74.3%. These included ST8, ST30, ST1, ST5 and ST88, suggesting a diverse population, harbouring multiple resistance and virulence genes, some of which have been previously associated with outbreaks in Ireland.

This study exposes a reservoir of MRSA in the community which may be imported into hospitals, leading to outbreaks. The diversity of MRSA lineages with enhanced virulence factors highlights the need for regular surveillance to ensure appropriate infection prevention and control interventions are implemented promptly.

McManus BA, Aloba BK, Earls MR, Brennan GI, O'Connell B, Monecke S, Ehrlich R, Shore AC, Coleman DC. Multiple distinct outbreaks of Panton-Valentine leucocidin-positive community-associated meticillin-resistant *Staphylococcus aureus* in Ireland investigated by whole-genome sequencing. *J Hosp Infect.* 2021 Feb; 108:72-80.

Abstract

Background: Panton-Valentine leucocidin (PVL)-positive community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA) is increasingly associated with infection outbreaks.

Aim: To investigate multiple suspected PVL-positive CA-MRSA outbreaks using whole-genome sequencing (WGS).

Methods: Forty-six suspected outbreak-associated isolates from 36 individuals at three separate Irish hospitals (H1-H3) and from separate incidents involving separate families associated with H2 were investigated by whole-genome multi-locus sequence typing (wgMLST).

Findings: Two clusters (CH1 and CH2) consisting of 8/10 and 6/6 PVL-positive t008 ST8-MRSA-IVa isolates from H1 and H2, respectively, were identified. Within each cluster, neighbouring isolates were separated by ≤ 5 allelic differences; however, ≥ 73 allelic differences were identified between the clusters, indicating two independent outbreaks. Isolates from the H3 maternity unit formed two clusters (CH3-SCI and CH3-SCII) composed of four PVL-negative t4667 ST5-MRSA-V and 14 PVL-positive t002 ST5-MRSA-IVc isolates, respectively. Within clusters, neighbouring isolates were separated by ≤ 24 allelic differences, whereas both clusters were separated by 1822 allelic differences, indicating two distinct H3 outbreaks. Eight PVL-positive t127 ST1-MRSA-V+fus and three PVL-negative t267 ST97-MRSA-V+fus isolates from two distinct H2-associated families FC1 (N = 4) and FC2 (N = 7) formed three separate clusters (FC1 (t127), FC2 (t127) and FC2 (t267)). Neighbouring isolates within clusters were closely related and exhibited ≤ 7 allelic differences. Intrafamilial transmission was apparent, but the detection of ≥ 48 allelic differences between clusters indicated no interfamilial transmission.

Conclusion: The frequent importation of PVL-positive CA-MRSA into healthcare settings, transmission and association with outbreaks is a serious ongoing concern. WGS is a highly discriminatory, informative method for deciphering such outbreaks conclusively.

Egan SA, Kavanagh NL, Shore AC, Mollerup S, Samaniego Castruita JA, O'Connell B, McManus BA, Brennan GI, Pinholt M, Westh H, Coleman DC. Genomic analysis of 600 vancomycin-resistant *Enterococcus faecium* reveals a high prevalence of ST80 and spread of similar *vanA* regions via IS1216E and plasmid transfer in diverse genetic lineages in Ireland. J Antimicrob Chemother. 2021 Nov 8: dkab393.

Abstract

Background: Vancomycin-resistant *Enterococcus faecium* (VRE_{fm}) cause a wide range of hospital infections. Ireland has had one of the highest invasive VRE_{fm} infection rates in Europe over the last decade, yet little is known about Irish VRE_{fm}.

Objectives: To investigate the population structure of Irish VRE_{fm}, explore diversity by analysing the *vanA* transposon region and compare Irish, Danish and global isolates.

Methods: *E. faecium* (n=648) from five Irish hospitals were investigated, including VRE_{fm} [547 rectal screening and 53 bloodstream infection (BSI)] isolates and 48 vancomycin-susceptible (VSE_{fm}) BSI isolates recovered between June 2017 and December 2019. WGS and core-genome MLST (cgMLST) were used to assess population structure. Genetic environments surrounding *vanA* were resolved by hybrid assembly of short-read (Illumina) and long-read (Oxford Nanopore Technologies) sequences.

Results: All isolates belonged to hospital-adapted clade A1 and the majority (435/648) belonged to MLST ST80. The population structure was highly polyclonal; cgMLST segregated 603/648 isolates into 51 clusters containing mixtures of screening and BSI isolates, isolates from different hospitals, and VRE_{fm} and VSE_{fm}. Isolates within clusters were closely related (mean average ≤16 allelic differences). The majority (96.5%) of VRE_{fm} harboured highly similar *vanA* regions located on circular or linear plasmids with multiple IS1216E insertions, variable organization of *vanA* operon genes and 78.6% harboured a truncated *tnpA* transposase. Comparison of 648 Irish isolates with 846 global *E. faecium* from 30 countries using cgMLST revealed little overlap.

Conclusions: Irish VRE_{fm} are polyclonal, yet harbour a characteristic plasmid-located *vanA* region with multiple IS1216E insertions that may facilitate spread.

Kinnevey PM, Kearney A, Shore AC, Earls MR, Brennan G, Poovelikunnel TT, Humphreys H, Coleman DC. Meticillin-resistant *Staphylococcus aureus* transmission among healthcare workers, patients and the environment in a large acute hospital under non-outbreak conditions investigated using whole-genome sequencing. J Hosp Infect. 2021 Dec; 118:99-107.

Abstract

Background: The role of meticillin-resistant *Staphylococcus aureus* (MRSA) colonization of healthcare workers (HCWs), patients and the hospital environment in MRSA transmission in non-outbreak settings is poorly understood.

Aims: To investigate transmission events (TEs) involving HCWs, patients and the environment under non-outbreak conditions in a hospital with a history of endemic MRSA using whole-genome sequencing (WGS).

Methods: HCW (N = 326) and patient (N = 388) volunteers on nine wards were tested for nasal and oral MRSA colonization over two years. Near-patient environment (N = 1164), high-frequency touch sites (N = 810) and air (N = 445) samples were screened for MRSA. Representative MRSA and clinical isolates were analysed by WGS and core-genome multi-locus sequence typing (cgMLST). Closely related isolates (≤ 24 allelic differences) were segregated into related isolated groups (RIGs).

Findings: In total, 155 MRSA were recovered: clinical isolates (N = 41), HCWs (N = 22), patients (N = 37), environmental isolates (N = 55). Nine clonal complexes (CCs) were identified among 110/155 MRSA sequenced with 77/110 assigned to CC22. Seventy-nine MRSA segregated into 17 RIGs. Numerous potential TEs were associated with CC22-MRSA (RIGs 1-15), CC45-MRSA (RIG-16) and CC8-MRSA (RIG-17). RIG-1, (the largest RIG) contained 24 ST22-MRSA-IVh from six HCWs, six patients, four clinical and eight environmental samples recovered over 17 months involving 7/9 wards. TEs involving HCW-to-patient, HCW-to-HCW, patient-to-patient and environmental contamination by HCW/patient isolates were evident. HCW, patient, clinical and environmental isolates were identified in four, nine, seven and 13 RIGs, respectively, with 12/13 of these containing isolates closely related to HCW and/or patient isolates.

Conclusions: WGS detected numerous potential hospital MRSA TEs involving HCWs, patients and the environment under non-outbreak conditions.

RESOURCES

Staff

During 2021 the staff working in the NMRSARL were:

- Gráinne Brennan
- Tanya Fleming
- Paul Grier
- Ciara Uí Mhuineachain
- Ludmila Fadejeva

The role of Director was discharged in an honorary capacity by Dr. Brian O'Connell, Consultant Microbiologist, SJH.

Administration

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

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.

Along with the Central Pathology Laboratory in SJH, NMRSARL has been involved in procuring a new computer system for several years and as part of this procurement, the special requirements of NMRSARL have been noted. However, all systems investigated to date would require extensive modification to accommodate NMRSARL's needs.

Finance

The budget allocated to the NMRSARL for the year to cover both pay and non-pay elements amounted to €325,000.

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