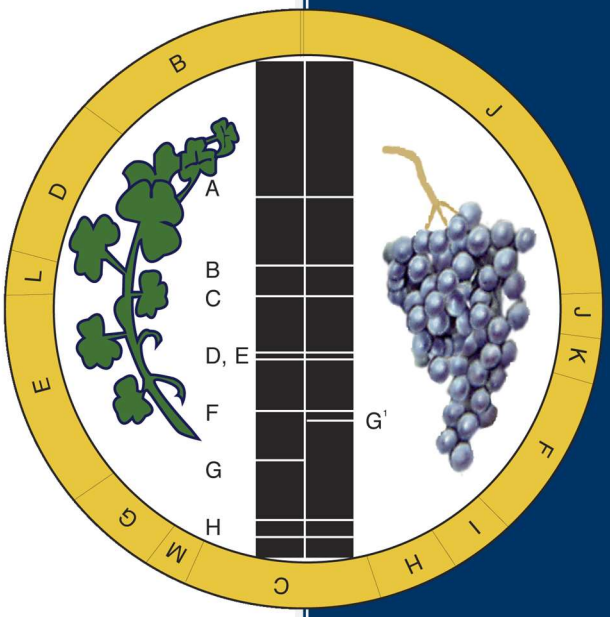
ANNUAL REPORT 2022



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 2022. The laboratory continued to deliver on its role in assisting medical professionals is the control of MRSA in hospitals and the community in Ireland and has seen a significant increase in requests in recent years due to an expansion in services offered.

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

Contents

SUMMARY

Public health impact	•The surveillance and identification of potential outbreaks of MRSA and MSSA •The laboratory also monitors the incidence of *pvl* carrying strains of *S. aureus* and the strains associated with healthcare infections •Surveillance of circulating strains and emergence of new strains of MRSA in Ireland
New service developments	•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	•During 2022, the EARS-Net project accounted for 9.2%% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for 46.8% •Further increase in the uptake of newer services including whole genome sequencing 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
Future developments	•The use of whole genome sequencing for outbreak investigation and characterisation of *S. aureus* 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 meticillin susceptible *S. aureus* (MSSA) isolates
  - For the detection of the *pvl* and exfoliative toxin genes
  - Outbreak investigation of strains using *spa* typing
- Epidemiological typing of *Enterococcus faecium* and detection of linezolid resistant determinants
- 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	99	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	662	Surveillance, recognition, investigation and management of PVL *S. aureus* in Ireland
MRSA & MSSA	Surveillance analysis and identification of trends	732	Typing and susceptibility testing of MRSA and MSSA isolates submitted throughout the year.
MRSA and MSSA and Enterococci	Surveillance	41	Outbreak/cluster investigations throughout Ireland
MRSA and MSSA	Confirmation of resistance against various antibiotic agents	933	Confirmation of resistance against glycopeptides, β-lactams, daptomycin and newer agents.
VRE and CoNS	Confirmation of linezolid and other resistance	193	Characterisation of resistance mechanism associated with increased resistance in VRE and CoNS

REFERENCE LABORATORY WORK

As expected MRSA isolates accounted for the largest proportion (47.4%) of the laboratory workload during 2022. Isolates recovered from bloodstream infections that were investigated under the European Antimicrobial Resistance Surveillance Network (EARS-Net) accounted for 9.2% of the overall workload and MRSA recovered from other sites being 38.2%. As in previous years the number of requests to investigate susceptible *S. aureus* and other Gram positive organisms have also increased now representing 36.2% and 16.2% respectively (Fig. 1).

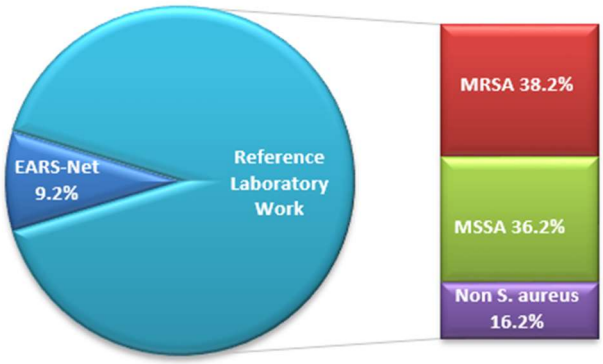


Fig 1 Workload of the NMRSARL during 2022

In recent years an increase in requests for investigations of MSSA isolates has led to a change in the services of the laboratory and 2022 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 genes (n=773) or *spa* typing (n=392). 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.

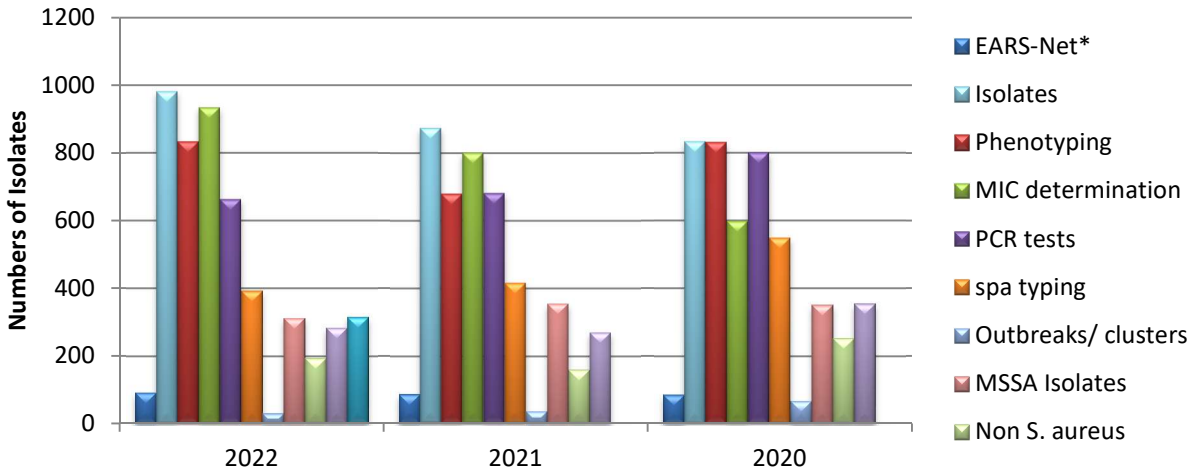


Fig 2 Distribution of workload throughout 2022

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. For over the last decade, the predominant strains circulating in Ireland was ST22-MRSA-IV and exhibits a non-multiantibiotic resistant susceptibility profile. However, in 2022 the prevalence of ST22-MRSA-IV decreased among the EARS-Net isolates and the other lineages recovered exhibited greater resistance to aminoglycosides and tetracyclines due to an increase in the number of possible community associated strains which are known to carry multiple virulence and resistance genes.

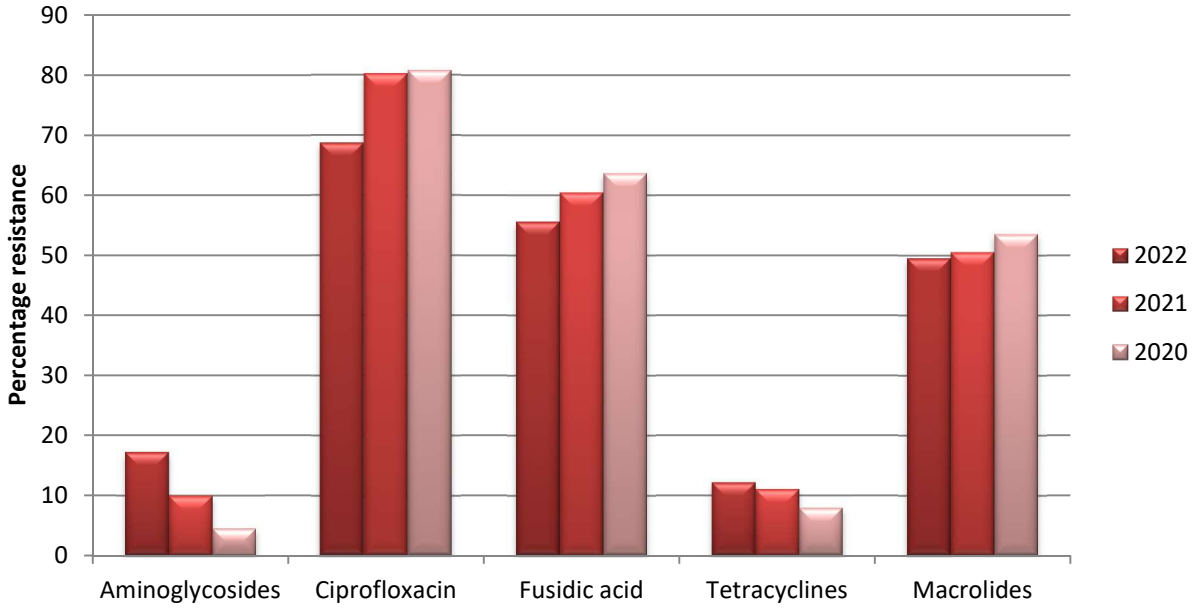


Fig 3 Resistance rates among EARS-Net isolates recovered in 2022

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. Whilst the ST22-MRSA-IV exhibits a non-multiantibiotic resistant profile many isolates recovered from non-BSI 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 60% of isolates exhibiting multi-antibiotic resistance, that is, resistance to three or more different classes of antibiotics.

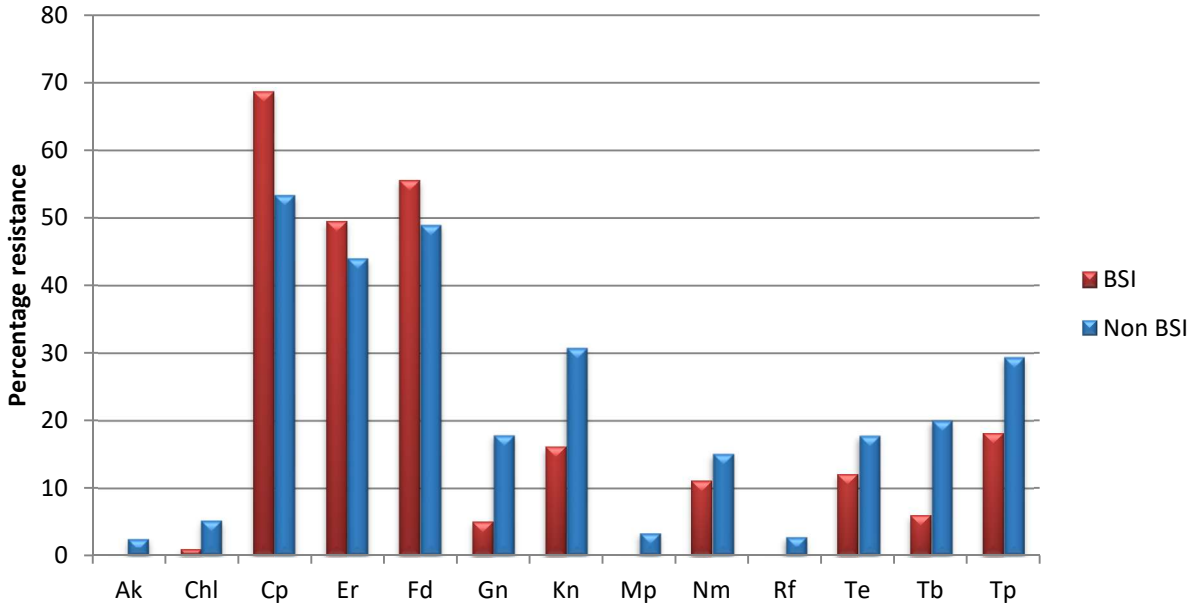


Fig 4 The percentage of blood stream MRSA isolates exhibiting resistance to each antimicrobial agent in comparison to those recovered from non-blood stream infections.

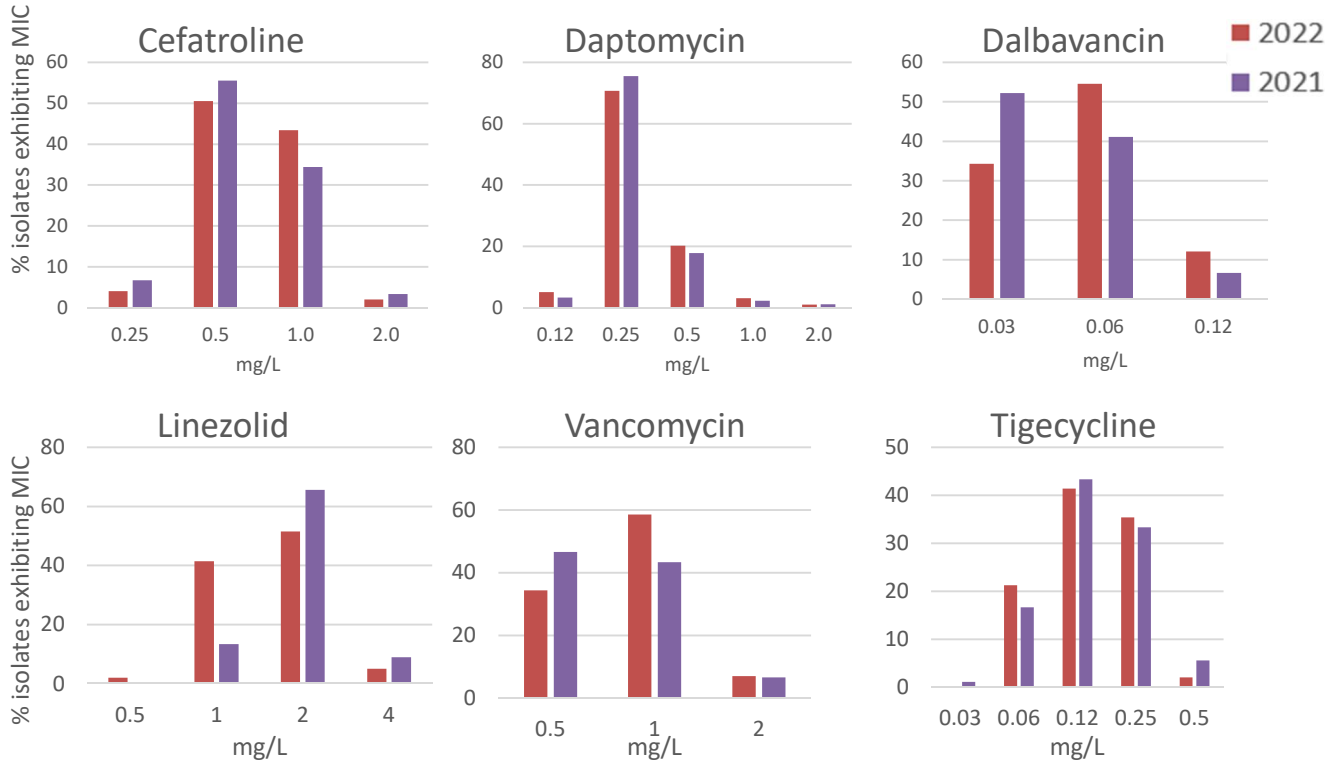
Resistance patterns determined for MRSA isolates by antibiogram- resistogram typing. Percentage for each agent includes those exhibiting resistance as determined in accordance with EUCAST or in-house developed interpretive criteria. Abbreviations: Ak; amikacin, Chl; chloramphenicol, Cp; ciprofloxacin, Er; erythromycin, Fd; fusidic acid, Gn; gentamicin, Kn; kanamycin, Mp; mupirocin, Nm; neomycin, Rf; rifampicin, 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 microdilutioninvestigation 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 brothmicrodilution 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.



Interpretive Criteria		
Agent	Susceptible <=	Resistant >
Ceftaroline	1.0	1.0
Tigecycline	0.5	0.5
Vancomycin	2.0	2.0
Dalbavancin	0.125	0.125
Linezolid	4.0	4.0
Daptomycin	1.0	1.0

Fig 5 The minimum inhibitory concentration (MIC) of MRSA isolates recovered from blood stream infections in 2022 compared to those recovered in 2021

Linezolid resistance among 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 2022, there were 50 isolates investigated for these resistance genes. Among isolates investigated 76% (38/50) exhibited phenotypic resistance and of these 4% (2/50) harboured *optrA* and 6% (3/50) were found to harbour *poxtA.* Detection of mutational resistance associated with 23S rRNA was also investigated the G2576T mutation was identified in 63.3% (38/60) of isolates. 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 the increasing in Ireland since the late 2000s (4).

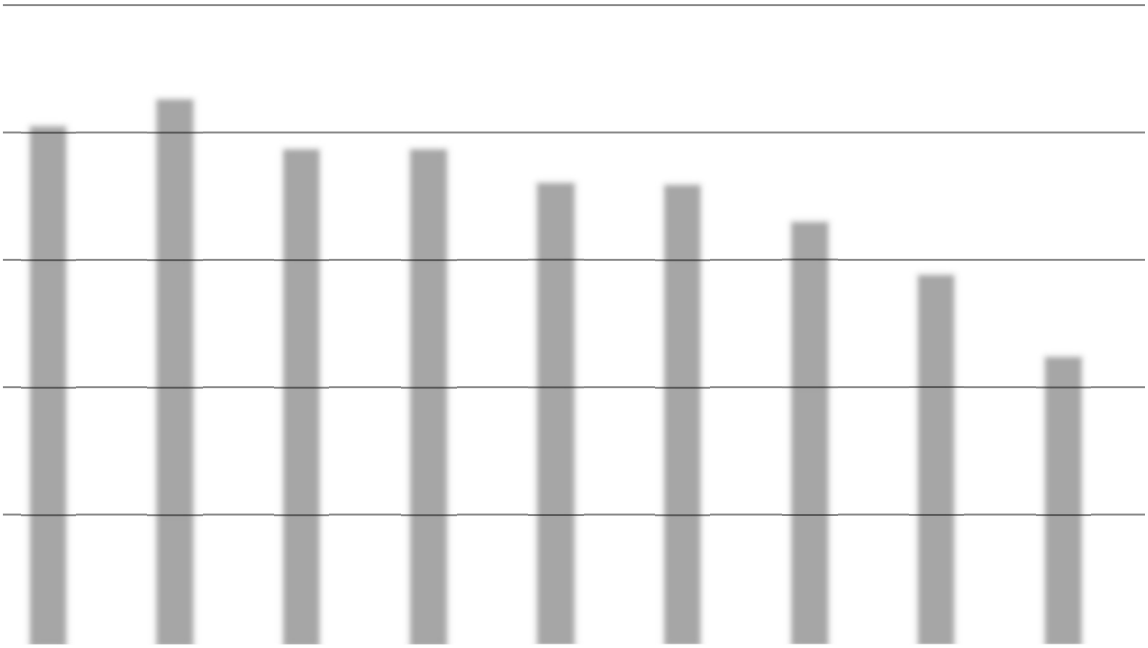


Fig 6 Epidemiological types of MRSA strains recovered from blood stream infections. 2014-2022. 2012-2018 MLST types inferred using *spa* typing and antibiogram resistogram (AR) typing.

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. Hence 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.

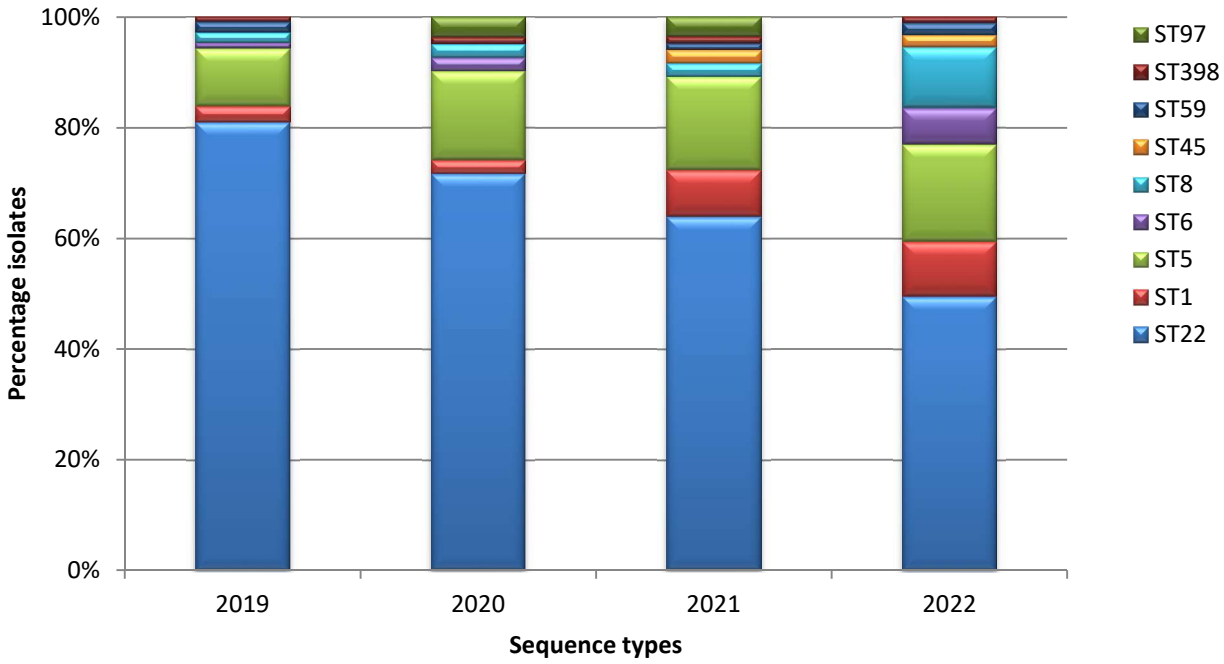


Fig 7 Epidemiological types of MRSA strains recovered from blood stream infection recovered in 2019-2022 investigated by whole genome sequencing analysis.

ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND

ST22-MRSA-IV is the pandemic clone in Ireland as it is in Europe but prevalence has been decreasing in prevalence in recent years and was associated with only 45.5% 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 2022 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.

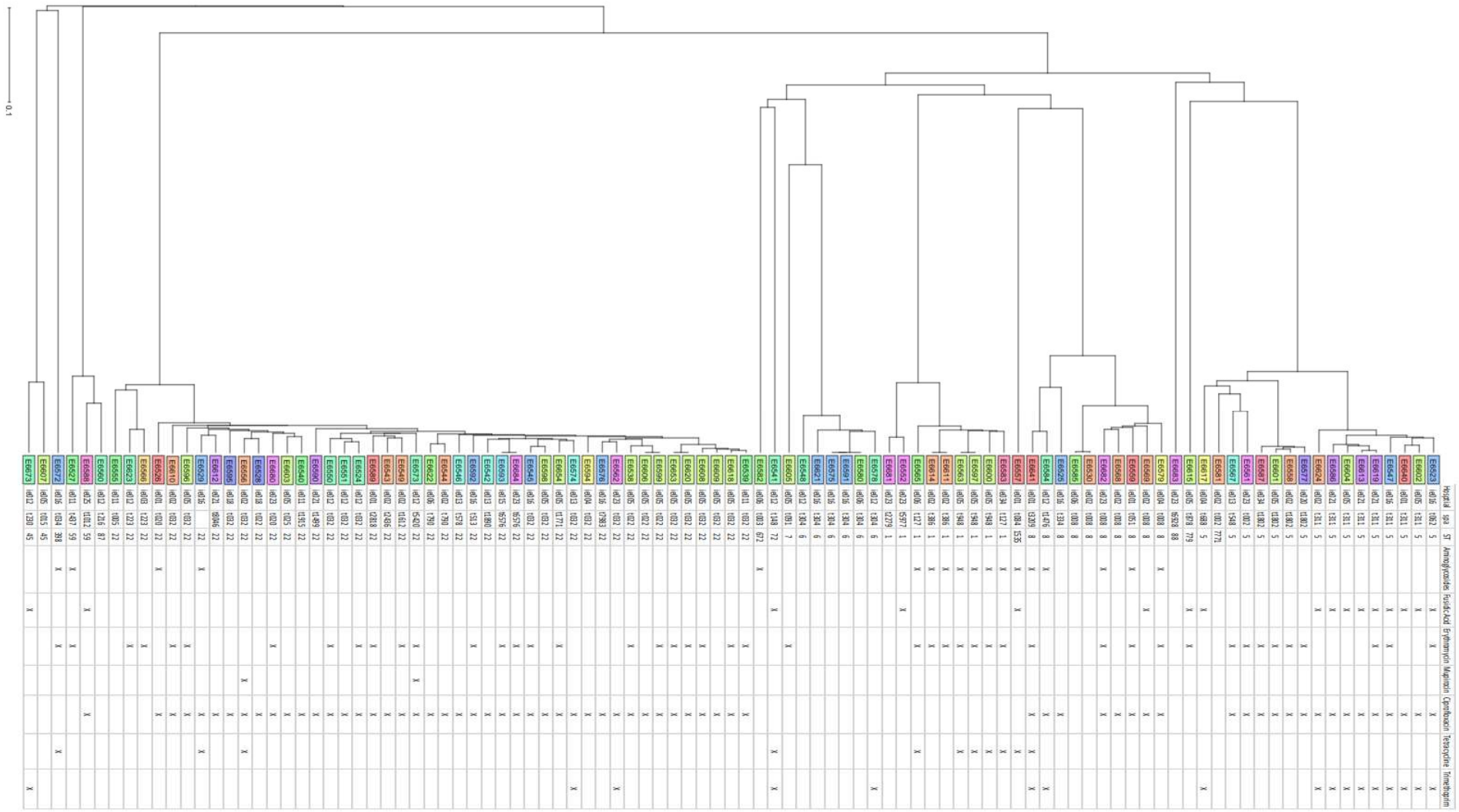


Fig 8 Phylogeny of MRSA isolates recovered from blood culture specimens during 2022. Coloured labels on tree represent different hospitals identified using their EARS-Net code. The phylogenetic tree was annotated with the distribution of selected resistance genes and where the gene was found to be present when there was >90% coverage of the gene at >30x depth of sequencing reads along with the *spa* type and MLST.

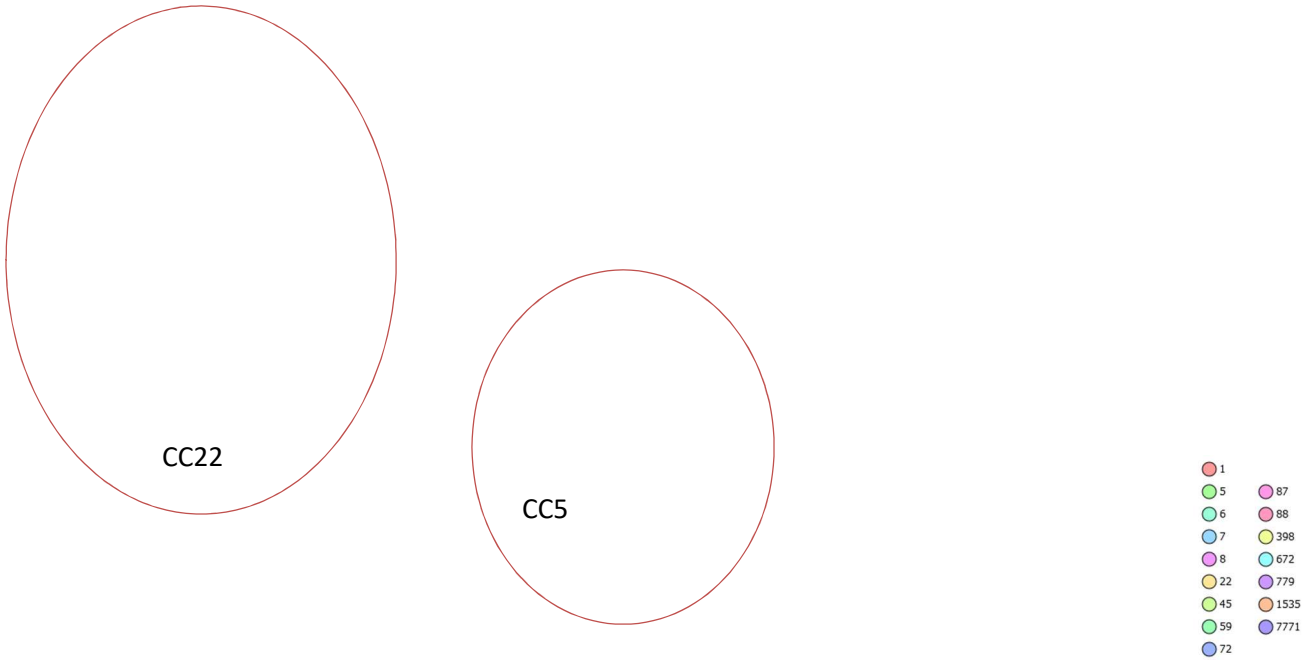


Fig 9: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all MRSA isolates recovered from blood stream infections during 2022. In each MST, MRSA isolates assigned to the same sequence type (ST) are indicated by separate colours. Closely related clusters 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. All clusters of closely related isolates were identified among those isolates assigned to CC22 and CC5.

Fig 10: A minimum spanning tree constructed from the ST22 MRSA isolates (n=53) recovered from blood stream infections in 2021. Seven distinct clusters were recognised involving 14 isolates. Each colour represents a different hospital. Three clusters (cluster 1, 3 and 6) included isolates recovered from two different hospitals (Cluster 1: IE005/ IE011 and Cluster 3/6: IE013/ IE023) while the remaining clusters were limited to isolates recovered within the same hospital. There was no further epidemiological data available regarding patient transfers between these hospitals.

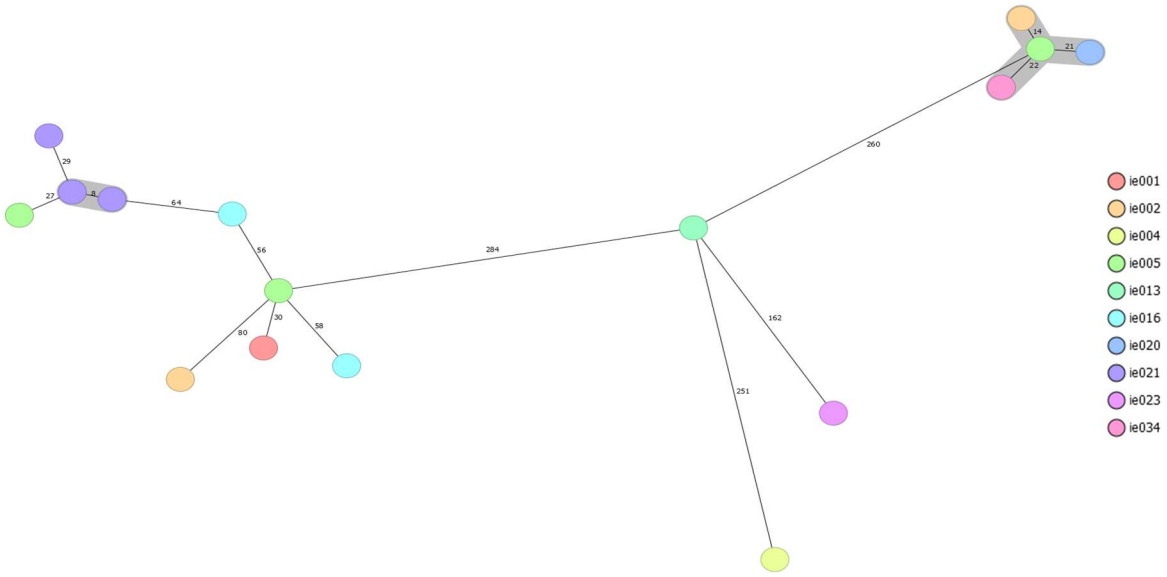


Fig 11: A Minimum spanning tree constructed from the ST5 MRSA isolates (n=14) recovered from blood stream infections in 2021. Two distinct clusters were recognised involving eight isolates. Each colour represents a different hospital. Cluster 1 involved isolates recovered from four different hospitals (IE012, IE023, IE034 and IE038) while Cluster 2 involved only isolates recovered from one hospital (IE012).

PVL positive *S. aureus*

Throughout 2022 the detection of PVL continued to be the most frequently requested test. The PVL toxin is a cytotoxigenic 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 2022, 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 22.0% (60/273) were found to be positive while 8.5% (26/307) of MSSA isolates were also positive.

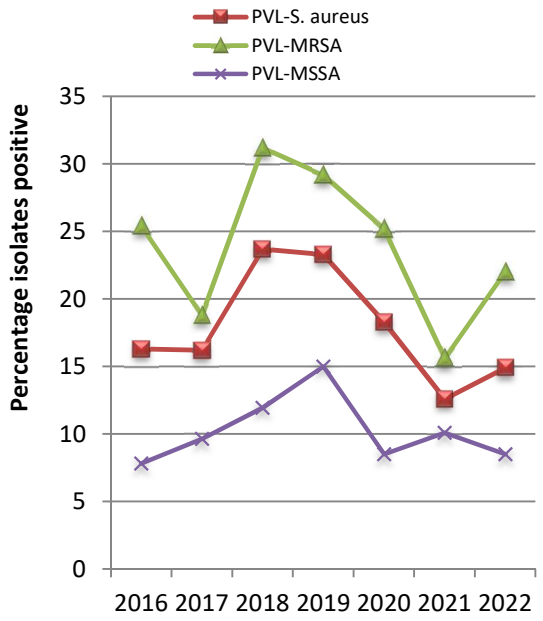


Fig 12: Frequency of PVL *S. aureus*

The change in the number of PVL-positive MRSA in recent 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 2022, 71% % of the isolates were limited to only eight sequence types with a further the 22% not assigned to any MLST by *spa* typing (Fig 13).

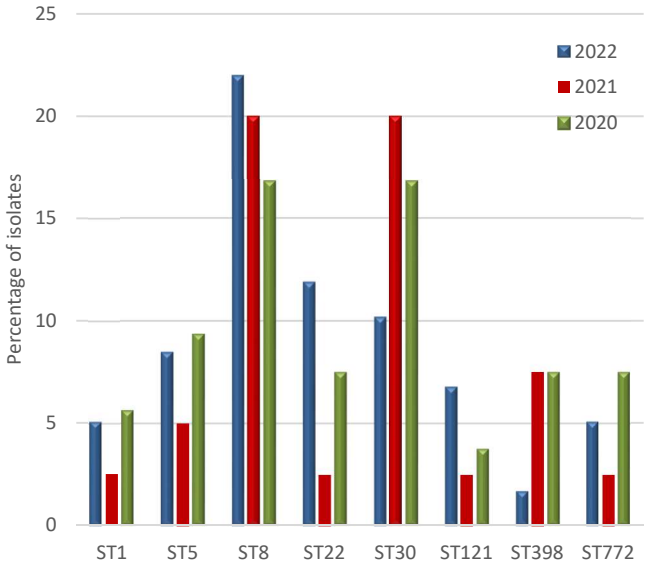


Fig 13 Distribution of sequence types among PVL-*S. aureus* isolates recovered in 2022.

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 14).

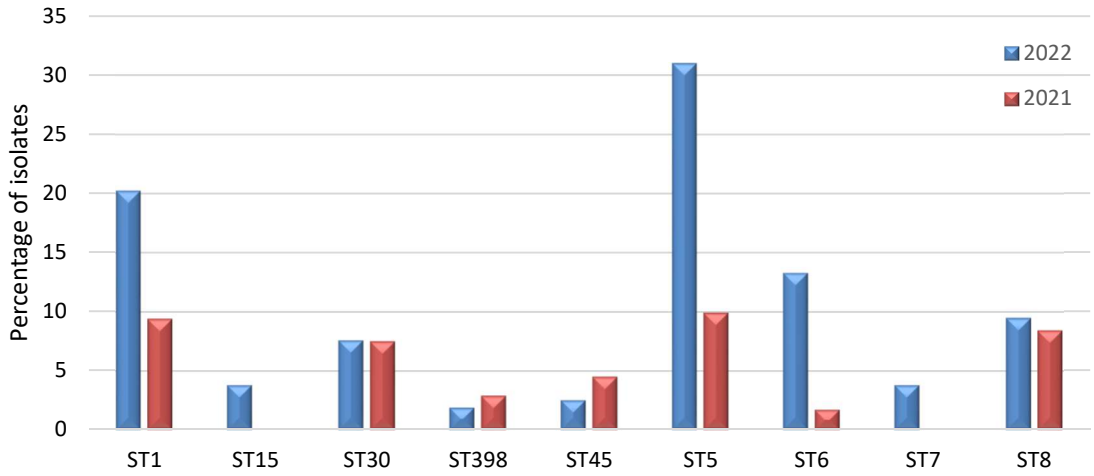


Fig 14 Most frequently recognised MLST among MRSA isolates investigated by *spa* typing during 2022

***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 NMRSASRL (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.

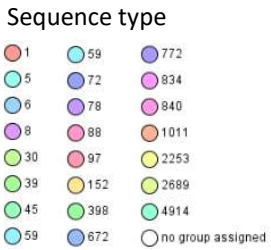


Fig 15: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all MRSA isolates associated with CA-MRSA lineages investigated by whole genome sequencing. In each MST, MRSA isolates assigned to the same sequence type (ST) are indicated by separate colours. 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.

WHOLE GENOME SEQUENCING TO INVESTIGATE VANCOMYCIN RESISTANT *ENTEROCOCCUS FAECIUM* RECOVERED IN IRISH HEALTHCARE SETTINGS

Enterococcus faecium, a resident of the gastrointestinal flora has been a persistent problem in Irish Healthcare settings for many years with vancomycin resistance exhibited by 28% of the isolates recovered from blood stream infections in 2022 (1). For several years the NMRSARL has collaborated with colleagues in the Dublin Dental Hospital to investigate the population structure of VR*Efm* from Irish hospitals using WGS, to explore diversity by investigating the vanA region and to identify potential characteristic features associated with Irish VR*Efm* in relation to the global populations (8).

This study found that all isolates were assigned to the hospital-adapted clade in ST80 but cgMLST assigned the isolates into 51 different clusters which included isolates from different hospitals and from both screening and blood stream infections, suggesting that the population is highly polyclonal. Within clusters, isolates were closely related (8). In investigating the *vanA* operon the study found that the majority of isolates harboured the highly similar *vanA* regions. A comparison of Irish isolates with an international collection showed very little overlap of populations (8).

In recent years the MRSARL has been assisting hospitals investigating outbreaks of VR*Efm* and in 2022 investigated 84 isolates from various different hospitals. The majority of isolates were assigned to ST80 and clonal complex CC17. Among the isolates there were 11 clusters recognized containing varying numbers of isolates. The remaining isolates were unrelated.

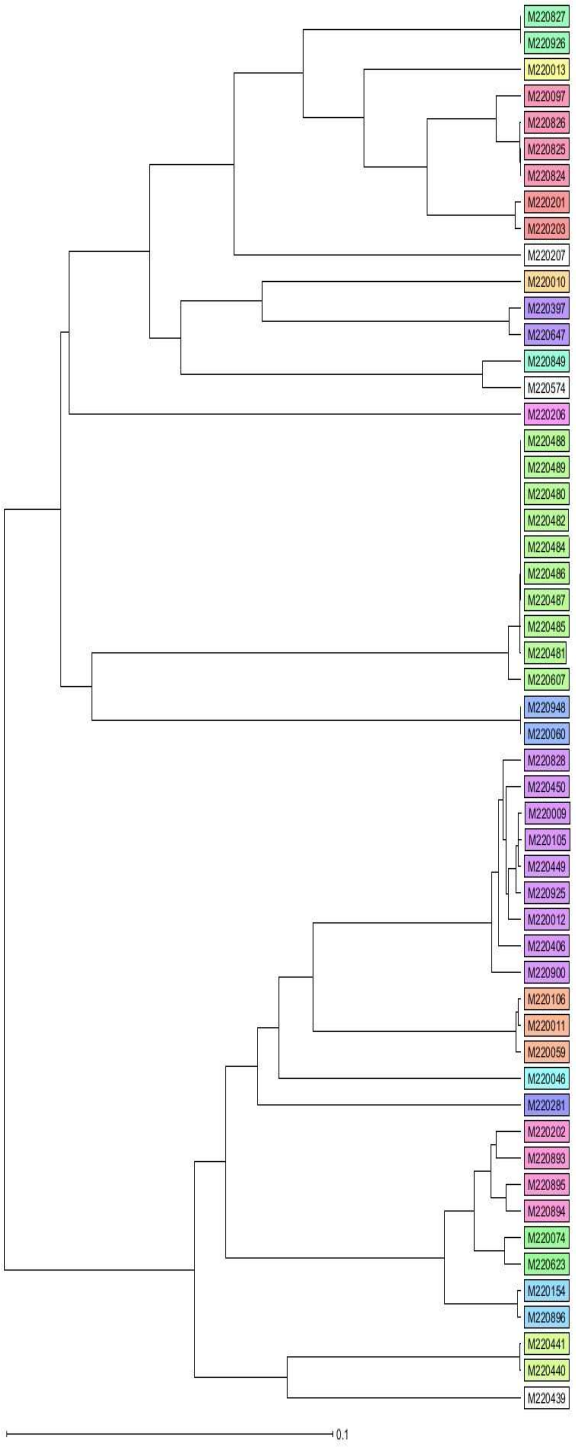


Fig 16 UPGMA tree of VREfm investigated during 2022 coloured based on clusters of isolates recognized.

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;

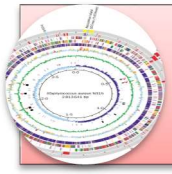
- Journal clubs
- Focus on Infection
- Antimicrobial Resistance
- Microbiology Advisory Body

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

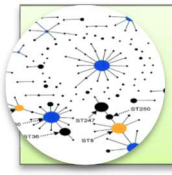
RESEARCH HIGHLIGHTS

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

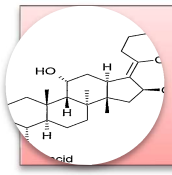
- Characterisation of the genotypes, virulence and antimicrobial resistance genes of *pvl*-positive MRSA in Ireland and MRSA in closed communities

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 decreasingMupirocin 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.

Kinnevey PM, Kearney A, Shore AC, Earls MR, Brennan GI, Poovelikunnel TT, Humphreys H, Coleman DC. Meticillin-susceptible *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. 2022 Sep; 127: 15-25.

Abstract

Background: The role of meticillin-susceptible Staphylococcus aureus (MSSA) colonization of healthcare workers (HCWs), patients and the hospital environment in MSSA transmission events (TEs) is poorly understood.

Aims: The role of meticillin-resistant Staphylococcus aureus (MRSA) was investigated recently under non-outbreak conditions in a large hospital with a history of endemic MRSA over 2 years using whole-genome sequencing (WGS). Numerous potential MRSA TEs were identified. The present study investigated MSSA TEs from the same sources during the same 2-year hospital study.

Methods: HCW (N=326) and patient (N=388) volunteers on nine wards were tested for nasal and oral MSSA colonization over 2 years. Near-patient environment (N=1164), high-frequency touch sites (N=810) and air (N=445) samples were screened for MSSA. Representative MSSA and clinical isolates were sequenced and analysed by core genome multi-locus sequence typing. Closely related isolates (≤24 allelic differences) were segregated into related isolate groups (RIGs). Potential TEs involving MSSA in RIGs from HCWs, patients and patient infections were identified in combination with epidemiological data.

Findings: In total, 635 MSSA were recovered: clinical isolates (N=82), HCWs (N=170), patients (N=120), and environmental isolates (N=263). Twenty-four clonal complexes (CCs) were identified among 406/635 MSSA sequenced, of which 183/406 segregated into 59 RIGs. Numerous potential HCW-to-patient, HCW-to-HCW and patient-to-patient TEs were identified, predominantly among CC5-MSSA, CC30-MSSA and CC45-MSSA. HCW, patient, clinical and environmental isolates were identified in 33, 24, six and 32 RIGs, respectively, with 19/32 of these containing MSSA related to HCW and/or patient isolates.

Conclusions: WGS detected numerous potential hospital MSSA TEs involving HCWs, patients and environmental contamination under non-outbreak conditions.

Aloba BK, Kinnevey PM, Monecke S, Brennan GI, O'Connell B, Blomfeldt A, McManus BA, Schneider-Brachert W, Tkadlec J, Ehricht R, Senok A, Bartels MD, Coleman DC. An emerging Panton-Valentine leukocidin-positive CC5-meticillin-resistant *Staphylococcus aureus*-IVc clone recovered from hospital and community settings over a 17-year period from 12 countries investigated by whole-genome sequencing. J Hosp Infect. 2023 Feb; 132:8-19. (accepted 2022)

Abstract

Background: A novel Panton-Valentine leukocidin (PVL)-positive meticillin-resistant *Staphylococcus aureus* (MRSA) clonal complex (CC)5-MRSA-IVc ('Sri Lankan' clone) was recently described from Sri Lanka. Similar isolates caused a recent Irish hospital outbreak.

Aim: To investigate the international dissemination and diversity of PVL-positive CC5-MRSA-IVc isolates from hospital and community settings using whole-genome sequencing (WGS).

Methods: Core-genome single nucleotide polymorphism (cgSNP) analysis, core-genome multi-locus sequence typing (cgMLST) and microarray-based detection of antimicrobial-resistance and virulence genes were used to investigate PVL-positive CC5-MRSA-IVc (N = 214 including 46 'Sri Lankan' clone) from hospital and community settings in 12 countries over 17 years. Comparators included 29 PVL-positive and 23 PVL-negative CC5/ST5-MRSA-I/II/IVa/IVc/IVg/V.

Results: Maximum-likelihood cgSNP analysis grouped 209/214 (97.7%) CC5-MRSA-IVc into Clade I; average of 110 cgSNPs between isolates. Clade III contained the five remaining CC5-MRSA-IVc; average of 92 cgSNPs between isolates. Clade II contained seven PVL-positive CC5-MRSA-IVa comparators, whereas the remaining 45 comparators formed an outlier group. Minimum-spanning cgMLST analysis revealed a comparably low average of 57 allelic differences between all CC5/ST5-MRSA-IVc. All 214 CC5/ST5-MRSA-IVc were identified as 'Sri Lankan' clone, predominantly spa type t002 (186/214) with low population diversity and harboured a similar range of virulence genes and variable antimicrobial-resistance genes. All 214 Sri Lankan clone isolates and Clade II comparators harboured a 9616-bp chromosomal PVL-encoding phage remnant, suggesting both arose from a PVL-positive meticillin-susceptible ancestor. Over half of Sri Lankan clone isolates were from infections (142/214), and where detailed metadata were available (168/214), most were community associated (85/168).

Conclusions: Stable chromosomal retention of pvl may facilitate Sri-Lankan clone dissemination.

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. 2022 Feb 2;77(2):320-330.

Abstract

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

Objectives: To investigate the population structure of Irish VR*Efm*, 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 VR*Efm* [547 rectal screening and 53 bloodstream infection (BSI)] isolates and 48 vancomycin-susceptible (VS*Efm*) 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 VR*Efm* and VS*Efm*. Isolates within clusters were closely related (mean average ≤16 allelic differences). The majority (96.5%) of VR*Efm* 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 VR*Efm* are polyclonal, yet harbour a characteristic plasmid-located *vanA* region with multiple IS1216E insertions that may facilitate spread.

RESOURCES

Staff

During 2022 the staff working in the NMRSARL were:

- Gráinne Brennan
- Tanya Fleming
- Paul Grier
- 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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