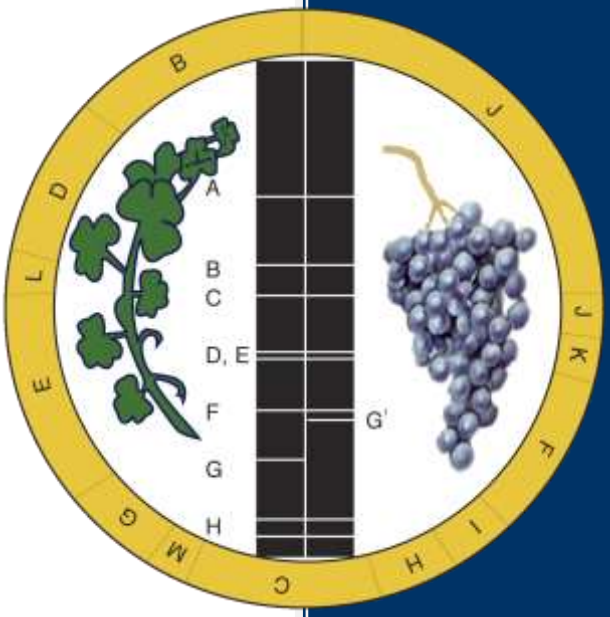
ANNUAL REPORT **2019**



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 2019. Throughout 2019, the laboratory continued to deliver on its role in assisting medical professionals is the control of MRSA in hospitals and the community in Ireland.

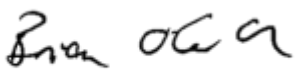
In addition to the routine work of the laboratory which includes molecular typing for epidemiological investigation and the detection of important virulence factors, service developments and projects during the year included:

- 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;
- Hosted an inspection team from the the European Centre for Disease Prevention and Control (ECDC) and the European Commission's Directorate General for Health and Food Safety who were assisting the competent authorities in the development and implementation of a national strategy for tackling antimicrobial resistance (AMR) based on a 'One Health' approach;
- 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.

Significant investment into microbiology laboratories within the St. James's Campus led to the purchase of a MiSeq which, once validated will enable the NMRSARL to 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

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UMMARY

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
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 2019, the EARS-Net project accounted for 12.1% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for 44.6% •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
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
- 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	120	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 *S. aureus* in Ireland
MRSA & MSSA	Surveillance analysis and identification of trends	807	Typing and susceptibility testing of MRSA and MSSA isolates submitted throughout the year.
Mupirocin resistant t127-MRSA-IV	Surveillance	1	Ongoing surveillance of multi-antibiotic resistant strain which was initially limited to one hospital but which has since spread to other hospitals and the community
MRSA and MSSA	Surveillance	288	Outbreak/cluster investigations (n=103) throughout Ireland
MRSA and MSSA	Confirmation of resistance against various antibiotic agents	622	Confirmation of resistance against glycopeptides, β-lactams, daptomycin and newer agents.
VRE and CoNS	Confirmation of linezolid resistance	106	Characterisation of resistance mechanism associated with increased linezolid resistance in VRE and CoNS
MSSA & MRSA	Characterisation of *S. aureus* recovered from healthcare workers	285	Determine prevalence of *S. aureus* among healthcare workers in Irish hospitals.
LA-MRSA	Surveillance	1	Characterisation of MRSA strains recovered from humans but with known association to livestock including isolates harbouring the *mecC* gene.

REFERENCE LABORATORY WORK

During 2019, work under the EARS-Net project accounted for 12.2% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for nearly 45% (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 2019 saw a further increase in the uptake of newer services including the investigation of linezolid resistance among Enterococci and CoNS.

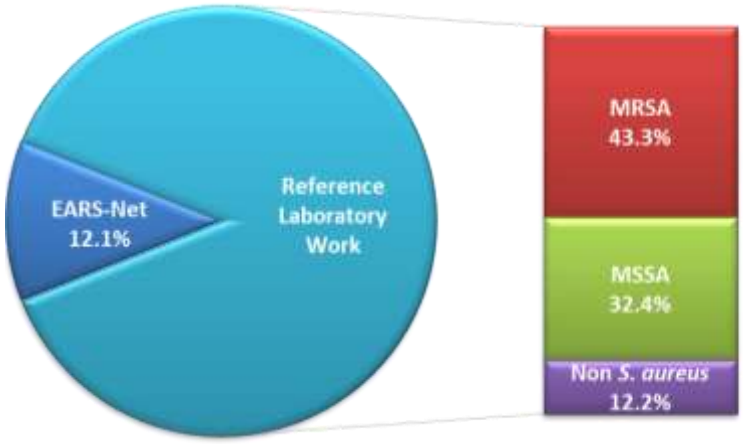


Fig 1 Workload of the NMRSARL during 2019

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=634) or *spa* typing (n=538). 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.

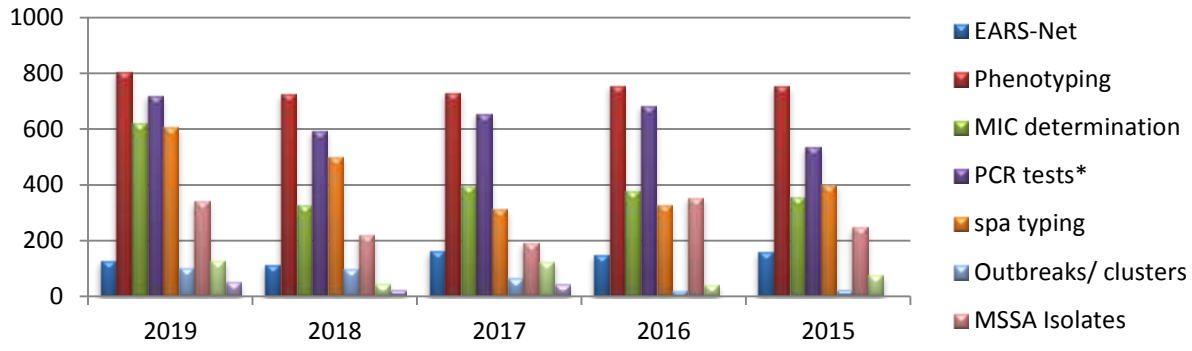


Fig 2 Distribution of workload throughout 2019

*In 2018 a further 339 *mecC* PCR tests were carried out. However during 2019 the NMRSARL changed the way *mec* PCR is performed a combined two PCR assays into a multiplex assay which allowed all isolates investigated for *mecA* to be investigated for *mecC* representing savings in staff time and laboratory reagents and consumables.

Linezolid resistance in Staphylococci and Enterococci

In 2019 Ireland had one of the highest proportion of vancomycin resistant *Enterococci faecium* (VRE*fm*) in Europe. In addition, in recent years an increase in resistance to linezolid has also been reported (1). Since 2016 the NMRSARL has investigated linezolid resistance in Enterococci and Staphylococci for the presence of *cfr* and *optrA* (2). Furthermore, following a report of an additional resistance gene *poxtA*, the NMRSARL retrospectively investigated all isolates submitted since 2018 for this gene (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 2019 there was a 3-fold increase in the number of isolates investigated for linezolid resistance including one outbreak caused by optrA-VRE*fm* (4). Among isolates investigated 69.8% (90/129) exhibited phenotypic resistance, 22 (17.1%) were found to harbour *optrA* and 1.6% (2/129) were positive for the *poxtA* gene. Mutational resistance associated with 23S rRNA were not investigated however an objective for the coming year is to validate an assay to test for the G2576T mutation.

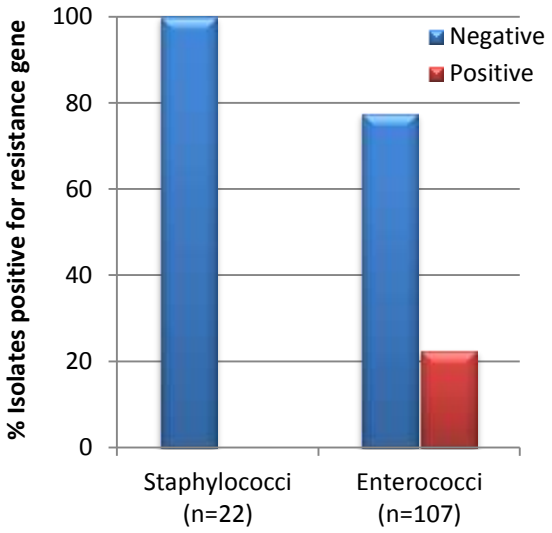


Fig 3 Distribution of linezolid resistant isolates investigated in 2019.

Since the introduction of screening for linezolid resistant genes in the NMRSARL, *optrA* and/or *poxtA* were identified in 20.7% (60/290) isolates, the highest prevalence reported to date. In conjunction with collaborators in the Dublin Dental University Hospital, the NMRSARL investigated the resistance mechanisms present in the linezolid resistant Enterococci isolates (5).

Among the isolates investigated, 30 *E. faecium* recovered from 11 hospitals were assigned to 10 STs using traditional MLST, with ST80 predominating. Using cgMLST seventeen *E. faecium* isolates were differentiated into seven clusters (CI–CVII) with clusters CI–CVI containing isolates of the same STs (ST17, ST787, ST789, ST202 and ST203). Furthermore some clusters contained isolates from the same hospitals while other consisted of isolates from two or more hospitals and a mixture of isolates exhibiting linezolid resistance associated with G2576T mutations or a resistance gene.

Among the *E. faecalis* recovered from 10 hospitals included in the study belonged to nine STs using traditional MLST, with ST480 predominating. Twenty of the *E. faecalis* isolates differentiated into four clusters (CI–CIV) using wgMLST with the remaining five isolates distantly related to any other isolate. Each cluster contained isolates from the same STs (ST6, ST21, ST480 and ST768). Only one cluster contained two isolates from the same hospital (H2) with the remaining clusters containing isolates from 2–8 hospitals.

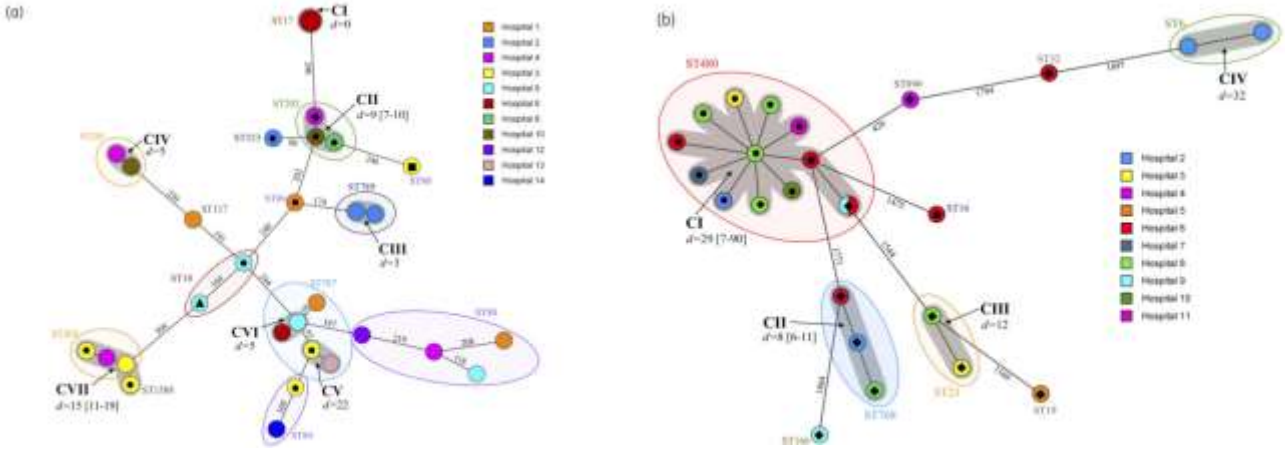


Fig 4 Minimum spanning trees based on (a) cgMLST data from 30 linezolid-resistant clinical *E. faecium* isolates and (b) wgMLST data from 25 linezolid-resistant *E. faecalis* clinical isolates. All isolates were recovered between June 2016 and August 2019 from 14 Irish hospitals, as denoted in the legends. The numbers on the branches represent the number of cgMLST/wgMLST allelic differences. STs are shown in coloured ovals. Grey shadowing around nodes indicates clusters of related isolates, which are labelled in bold and denoted CI–VII; 'd=' values indicate average allelic differences and the range in square brackets. Isolate designations are as follows: filled black circle, poxtA positive; filled black diamond, optrA positive; filled black square, optrA positive and cfr(D) positive; and filled black triangle, optrA positive and poxtA positive. Isolates not marked with a symbol were negative for linezolid resistance genes and harboured various copy numbers (1–5) of the G2576T 23S mutation associated with linezolid resistance.

PVL positive *S. aureus*

Throughout 2019 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 2019, 634 *S. aureus* isolates (non-BSI) were investigated for carriage of the *lukS-PV* and *lukF-PV* genes encoding for PVL and representing an increase of 19.2% from 2018. The isolates investigated included 356 MRSA and 278 MSSA.

Among the MRSA isolates 31.2% (102/327) were found to be positive while 11.8% (24/202) of MSSA isolates were also positive.

The significant increase in the number of PVL-positive MRSA during 2018 was primarily due to an increase in the number of outbreaks and clusters in healthcare settings identified during that year.

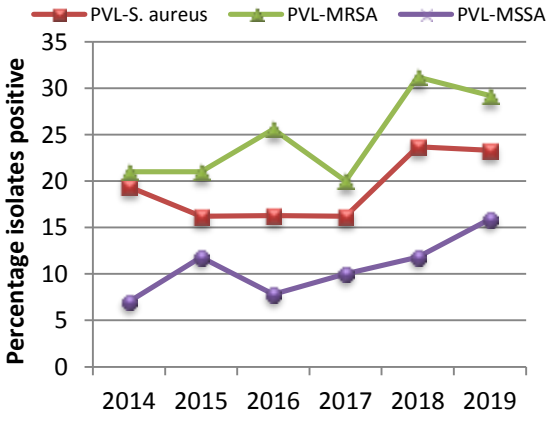


Figure 5 Percentage of isolates harboring PVL in 2019

As in previous years, the distribution of epidemiological types among PVL-*S. aureus* is limited with less diversity seen among the MRSA isolates. However, while 63% of the MRSA isolates investigated in 2018 were associated with only three types (ST8, ST30 and ST5), in 2019 these STs accounted for only 48% of the total collection.

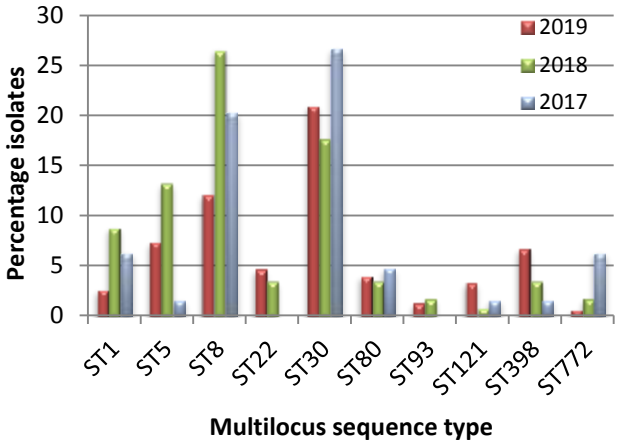


Figure 6 Distribution of sequence types among PVL-*S. aureus* isolates recovered in 2019.

Both MRSA and MSSA were found to be associated with only four STs (ST5, ST30, ST398 and ST80). There was a further increase in the frequency at which ST398-PVL *S. aureus* observed having increased from 1.6% in 2017 to 6.8% in 2019. Reports of PVL-positive ST398 are still infrequent and where present are often associated with severe skin and soft tissue infections often with epidemiological links to .South East Asia.

Among the PVL-*S. aureus* isolates there were 11 clusters involving 25 patients, of which seven involved inpatients while the remaining four were in the community.

Among the isolates recovered from blood stream infections only 5% were PVL positive (5/120) however all of these were assigned to different MLST.

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.

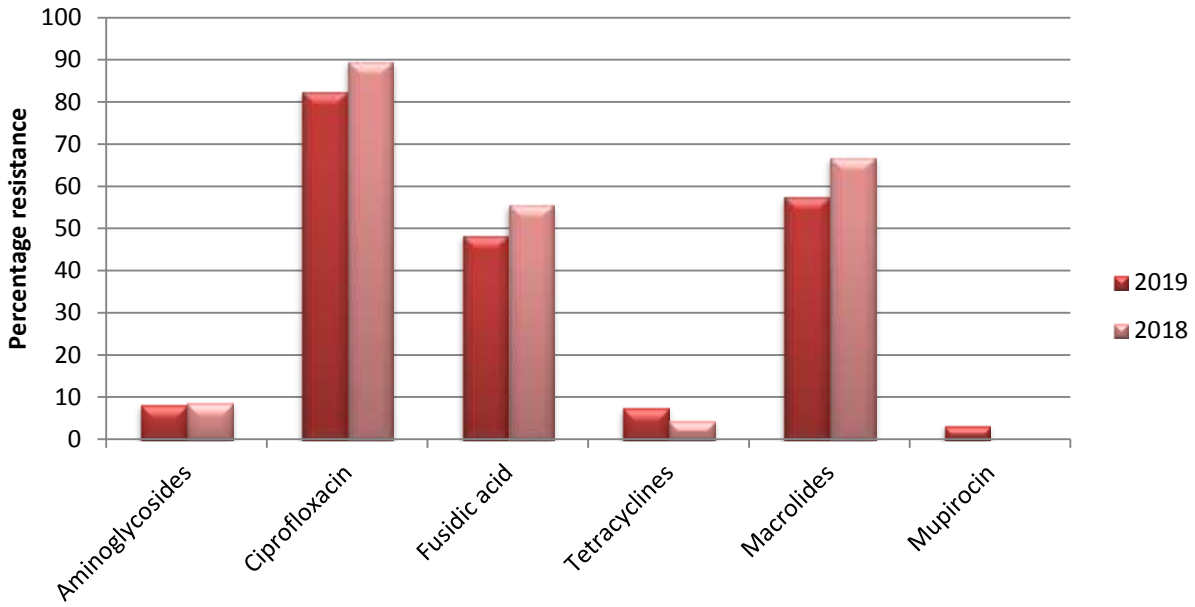


Fig 7 Resistance rates among EARS-Net isolates recovered in 2019

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 74% 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.

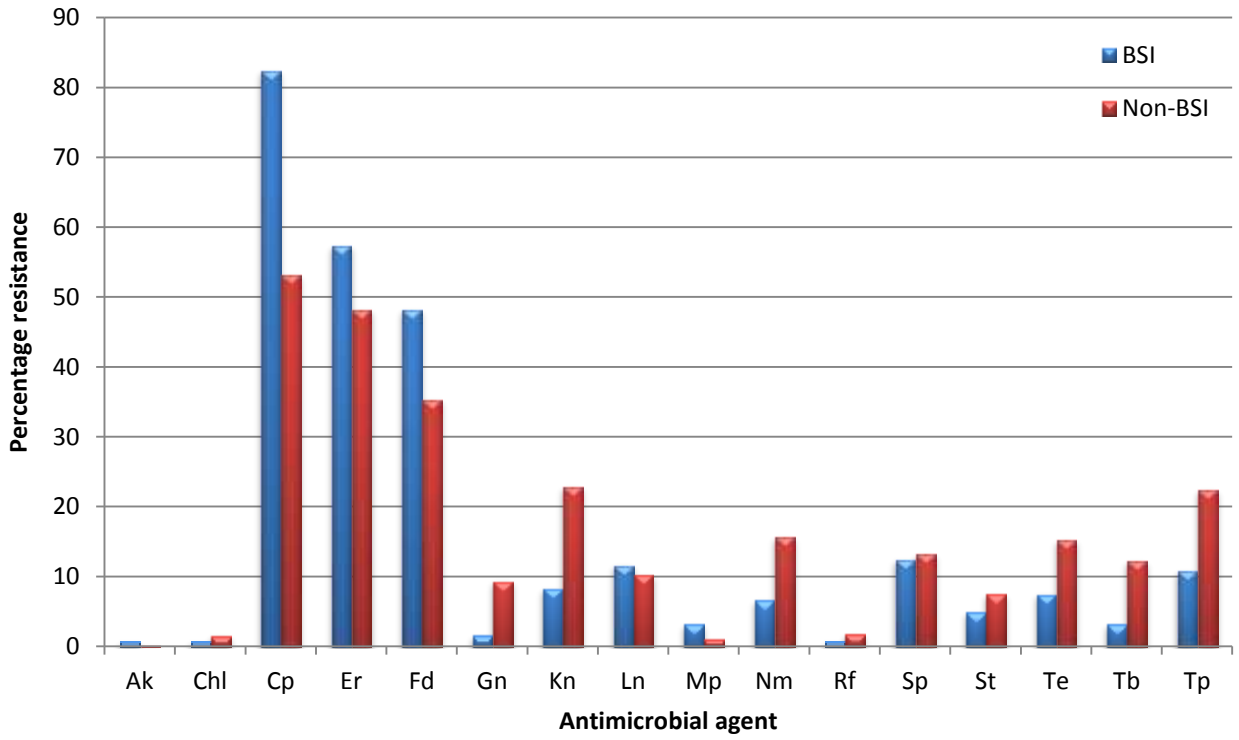


Fig 8 The percentage of blood stream MRSA isolates exhibiting resistance to each antimicrobial agent in comparison to those recovered from non-blood stream infections.

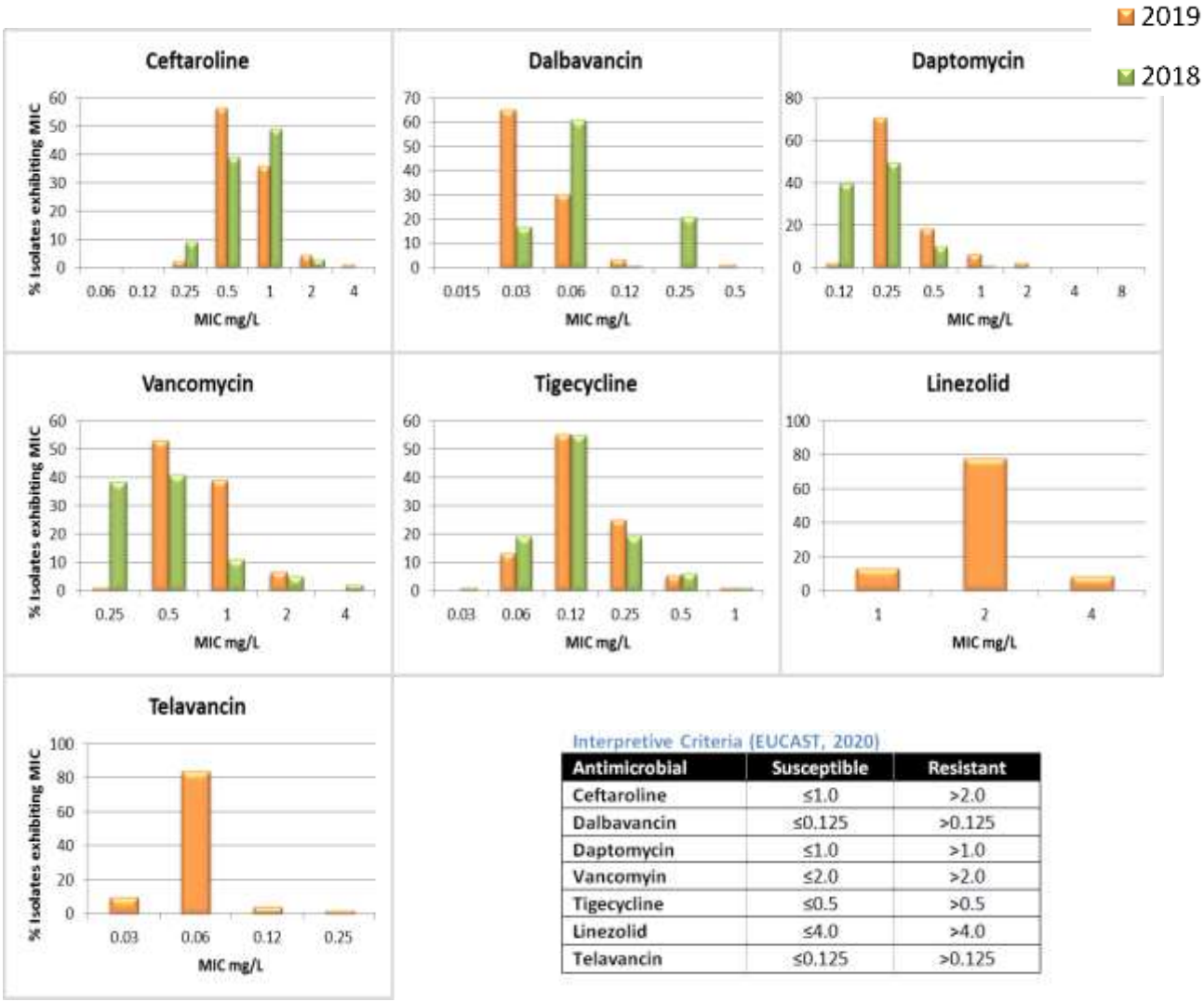
Resistance patterns determined for MRSA isolates by antibiogram- resistogram typing. Percentage for each agent includes those exhibiting resistance as determined in accordance with EUCAST or in-house developed interpretive criteria. Abbreviations: Ak; amikacin, Chl; chloramphenicol, Cp; ciprofloxacin, Er; erythromycin, Fd; fusidic acid, Gn; gentamicin, Kn; kanamycin, Mp; mupirocin, Nm; neomycin, 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 brothmicrodilution on a selection of 100 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 2018 determined using gradient MIC strips. Linezolid and telavancin were not previously monitored however results for the distribution of MIC of isolates in 2019 are shown.



Interpretive Criteria (EUCAST, 2020)

Antimicrobial	Susceptible	Resistant
Ceftaroline	≤1.0	>2.0
Dalbavancin	≤0.125	>0.125
Daptomycin	≤1.0	>1.0
Vancomyin	≤2.0	>2.0
Tigecycline	≤0.5	>0.5
Linezolid	≤4.0	>4.0
Telavancin	≤0.125	>0.125

EPIDEMIOLOGICAL TYPING OF MRSA IN IRELAND

For a number of years the NMRSARL has used phenotypic and molecular epidemiological typing techniques. Molecular techniques includes *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. In 2019 however, all isolates submitted to the NMRSARL for investigation under the EARS-Net project underwent whole genome sequencing.

Whole genome sequencing (WGS) found that, similar to previous years, ST22-MRSA-IV continues to be the predominant strain circulating in healthcare settings. 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 (6).

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, ST6, ST8, ST30, ST59, ST72, ST152, ST398, ST834, ST2250, ST3136. 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 South East Asia;
- ST2250 is associated with *S. argenteus* which is rarely identified in laboratories due to the close similarities to *S. aureus* and guidance not to distinguish these strains due to similar pathogenicities.

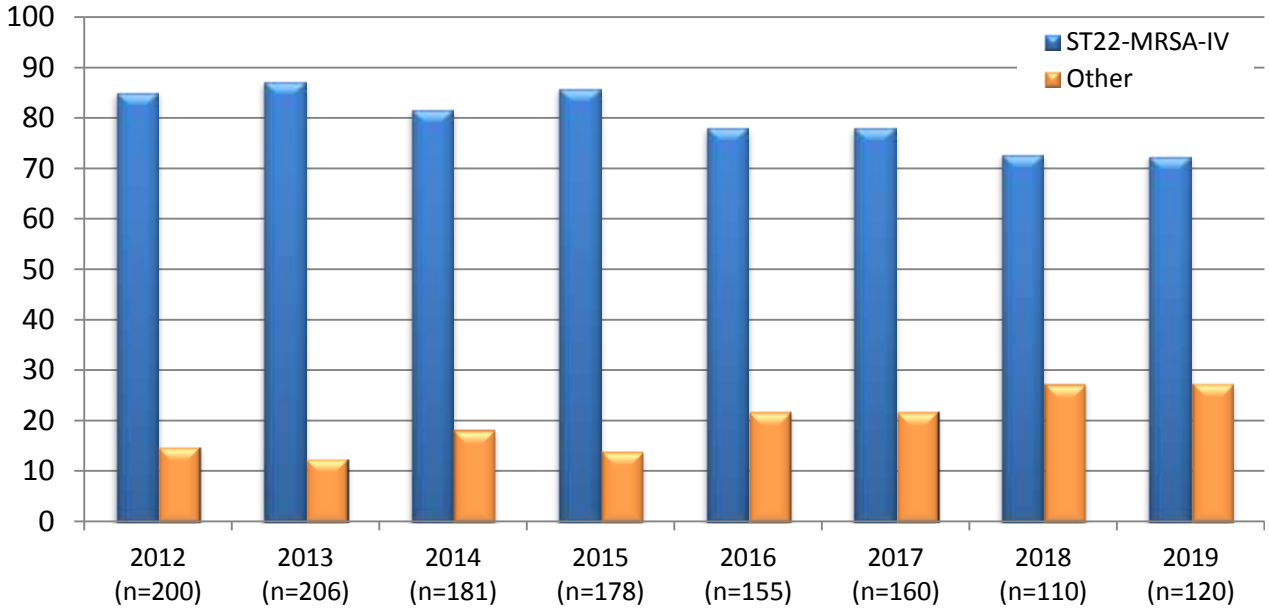


Fig 9 Epidemiological types of MRSA strains recovered from blood stream infections. 2012-2018 MLST types inferred using *spa* typing and antibiogram resistogram (AR) typing. MLST assigned to isolates investigated in 2019 following whole genome sequencing analysis. The total number of isolates investigated each year is shown in parentheses.

ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND

Like Europe, ST22-MRSA-IV is the pandemic clone in Ireland and, in 2019, was associated with 73.7% 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 (6).

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 (6).

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=83) recovered from blood cultures during 2019.

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 2019 EARS-Net collection, there were fourteen occasions where isolates had fewer than 24 differences however, and often these were from different hospitals. When comparing isolates from the same hospital there were was only one incident of ≤24 snp differences within one hospital indicating ancestral relatedness among the two isolates (E6339 and E6340) however the analysis of the mobile SCC*mec* element found it to be SCC*mec*IV(2B) and SCC*mec*IVc(2B) along with E6339 harbouring more resistance determinants that E6340.

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 10.)

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-lacamase), *erm*(C)/*lnu*(A) (macrolides) and *ant*(4)/*aph*(2) (aminoglycosides) (Fig 10). 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*).

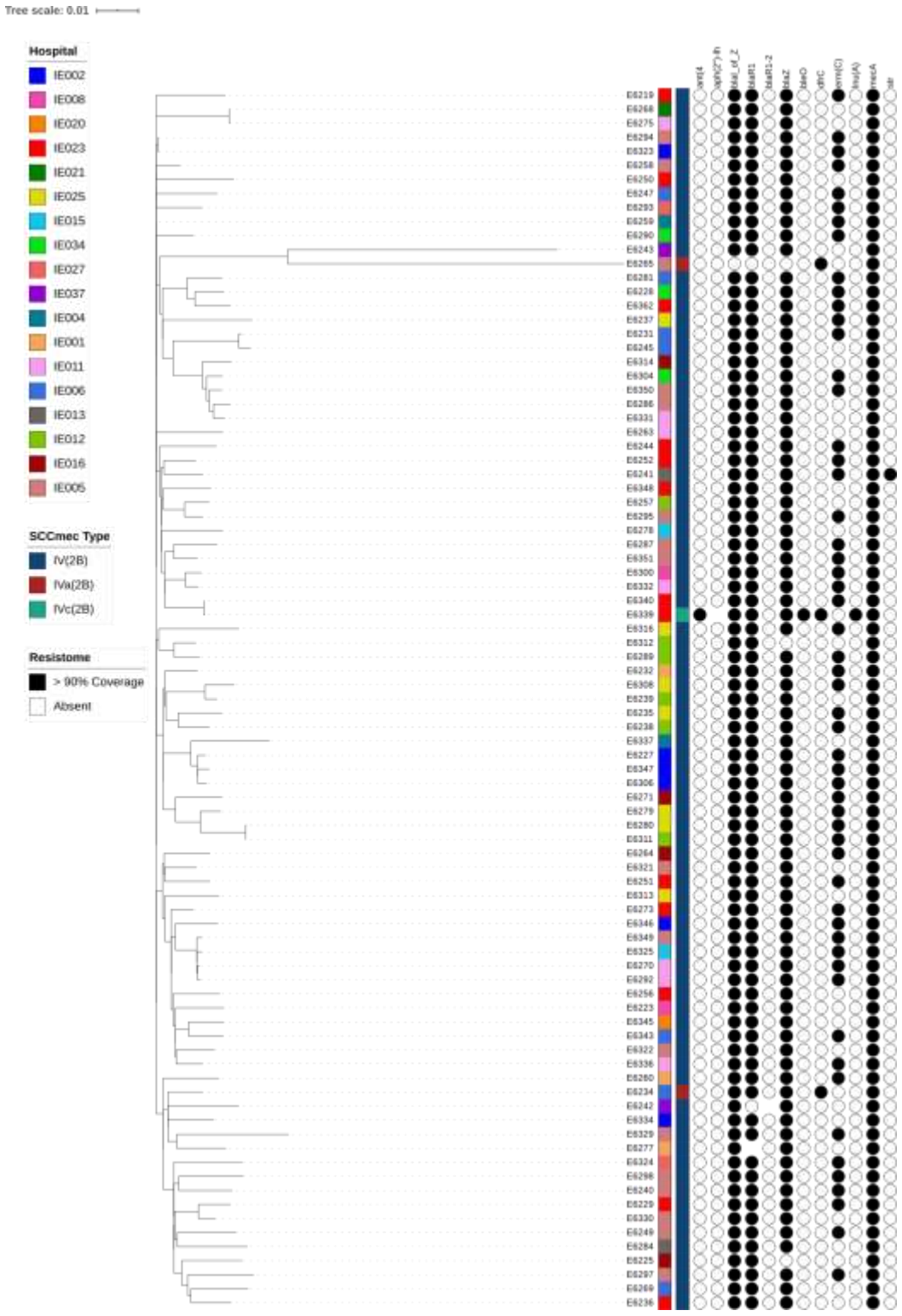


Fig 10 Phylogeny of MRSA ST22-MRSA-IV isolates. A rooted maximum-likelihood phylogenetic tree was annotated with the distribution of selected resistance genes and SCC*mec* elements present where the gene was found to be present when there was >90% coverage of the gene at >30x depth of sequencing reads.

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. Due to the emergence of newer MRSA strains with increased diversity, the NMRSARL has increased the number of isolates undergoing molecular 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 (7) .

Using the inferred MLST data available from the *spa* typing online database the most frequently recognised MLST types accounted for over 40% of the isolates and, similar to previous years, included ST1, ST5, ST8 and ST30 (Fig 10). The declining trend in the frequency of ST1 and increase in ST5 seen in 2018 continued in 2019. Similarly a further increase in the number of ST398 and ST30 was observed.

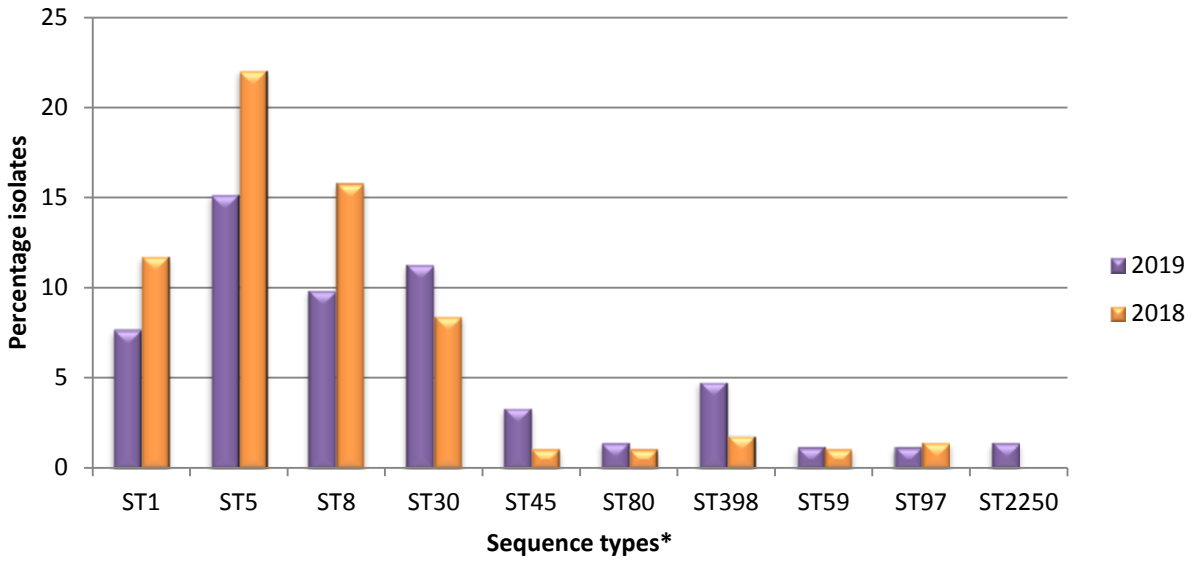


Fig 10 Most frequently recognised MLST among MRSA isolates investigated by *spa* typing during 2019

***Sequence type inferred from data available on the Ridom *spa* typing database. Inferred MLST were not available for 32% of *spa* types (n=192) recognised.**

WHOLE GENOME SEQUENCING TO INVESTIGATE OUTBREAKS CAUSED BY CA-MRSA LINEAGES

In recent years the NMRSARL has been involved in a number of studies investigating the emergence of different lineages of MRSA in Ireland in in particular those which have been associated with outbreaks in healthcare facilities(8). During 2019 these studies continued focusing in particular on strains causing outbreaks in healthcare facilities. These have included:

- PVL positive t002-CC5-MRSA-IV (n=9) causing a prolonged outbreak in a maternity ward;
- 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). These isolates were also compared to isolates recovered from the community during the same period to determine if there was anyone further transmission out of the healthcare setting. Whole genome sequencing showed that, while isolates recovered from the within each hospital were related to each other, they were unrelated to isolates recovered in the other hospital and in the community

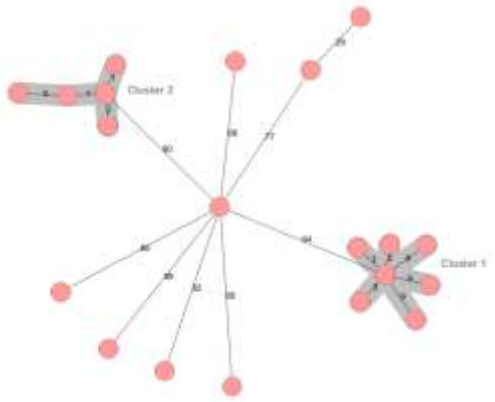


Figure 11 Minimum spanning tree constructed from cgMLST of PVL positive t008-ST8-MRSA-IV recovered from clusters in two healthcare facilities and the community.

- PVL positive t127-ST1-MRSA-V recovered from a previously MRSA negative patient during a prolonged stay within a hospital. Further investigations led to the recovery of isolates from outside the healthcare setting and sequencing showed these isolates to be indistinguishable to that recovered from the in-patient. This strain had previously been associated with several other clusters in the community and so was compared to those isolates along with other strains recovered within the ward in which the patient was admitted.

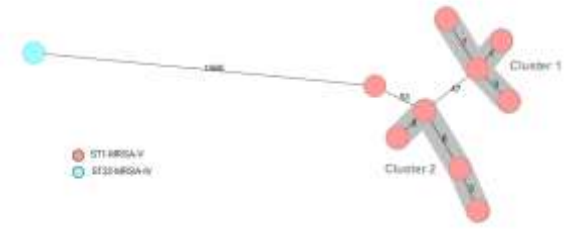


Figure 12 Minimum spanning tree constructed from cgMLST of ST1-MRSA-V recovered from the community in Ireland compared to ST22-MRSA-IV, a typical healthcare associated MRSA in Ireland.

We would like to thank collaborators in the Dublin Dental University Hospital for performing the WGS throughout the year and for colleagues in the IMRL in assisting in data interpretation.

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 2019 the NMRSARL confirmed the presence of *mecC* in two isolates, both of which were recovered from humans in Dublin. Elsewhere *mecC* has frequently been associated with MRSA recovered from animal sources; however in Ireland has only once been recovered from 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.

In contrast however, PVL-positive ST398 has continued to increase in prevalence in Ireland. This strain is frequently reported from South East Asia and many of the cases seen here have epidemiological links to that region.

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.

The laboratory also assisted in a number of post graduate students undertaking projects including epidemiological typing of MRSA recovered from a 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 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

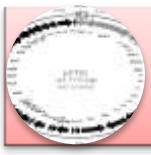
- Characterisation of the genotypes, virulence and antimicrobial resistance genes of *pvl*-positive MRSA in Ireland and MRSA in closed communities

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

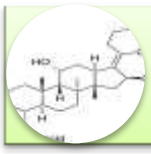
MSSA

- 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.

Eike J. Steinig *et al.,* Evolution and global transmission of a multidrug-resistant, community-associated MRSA lineage from the Indian subcontinent. *Clin Sci Epidemiol*., **10;** 6: e01105-19

Abstract

The evolution and global transmission of antimicrobial resistance have been well documented for Gram-negative bacteria and health care-associated epidemic pathogens, often emerging from regions with heavy antimicrobial use. However, the degree to which similar processes occur with Gram-positive bacteria in the community setting is less well understood.

In this study, we traced the recent origins and global spread of a multidrug-resistant, community-associated *Staphylococcus aureus* lineage from the Indian subcontinent, the Bengal Bay clone (ST772). We generated whole-genome sequence data of 340 isolates from 14 countries, including the first isolates from Bangladesh and India, to reconstruct the evolutionary history and genomic epidemiology of the lineage. Our data show that the clone emerged on the Indian subcontinent in the early 1960s and disseminated rapidly in the 1990s.

Short-term outbreaks in community and health care settings occurred following intercontinental transmission, typically associated with travel and family contacts on the subcontinent, but ongoing endemic transmission was uncommon. Acquisition of a multidrug resistance integrated plasmid was instrumental in the emergence of a single dominant and globally disseminated clade in the early 1990s. Phenotypic data on biofilm, growth, and toxicity point to antimicrobial resistance as the driving force in the evolution of ST772.

The Bengal Bay clone therefore combines the multidrug resistance of traditional health care-associated clones with the epidemiological transmission of community-associated methicillin-resistant *S. aureus* (MRSA). Our study demonstrates the importance of whole-genome sequencing for tracking the evolution of emerging and resistant pathogens. It provides a critical framework for ongoing surveillance of the clone on the Indian subcontinent and elsewhere.

Deasy EC, Brennan GI, Tecklenborg SC, Umeh C, Coleman DC, Shore AC. A molecular epidemiological investigation of methicillin-susceptible *Staphylococcus aureus* causing bloodstream infections in Ireland, 2006-2017. *Eur J Clin Microbiol Infect Dis*. **38**: 5; 927-936

Abstract

The prevalence of methicillin-susceptible Staphylococcus aureus (MSSA) bloodstream infections (BSIs) has increased in many countries, including Ireland. This study aimed to investigate the molecular epidemiology of MSSA causing BSIs in Irish hospitals between 2006 and 2017, when MSSA BSIs increased, to identify any potential patient or pathogen contributing factors.

A total of 252 MSSA isolates from patients in Irish hospitals in 2006/2007, 2011 and 2017 underwent *spa* typing and DNA microarray profiling. Each patient's gender, age, 14-day mortality and epidemiological context of infection were recorded. Significant increases in community-onset (CO) MSSA BSIs and the average patient's age and decreases in hospital-onset (HO) MSSA were identified. Although, extensive genetic diversity was detected amongst the isolates, i.e. 24 multilocus sequence type clonal complexes (CCs)/sequence types and 124 *spa* types, three CCs (CC30, CC45, CC5) dominated, albeit in different proportions, during the study periods. CC30 declined significantly, in particular spa type t021, and was more common amongst HO-MSSA and CC45 and CC8 increased, particularly spa types t015 and t008, respectively, and were more common amongst CO-MSSA. Five of the seven most frequent *spa* types were more common amongst CO-MSSA.

Although overall multidrug resistance decreased, the prevalence of *erm*(C) increased significantly and virulence genes decreased, mostly notably *egc*, *tst*, *scn*, *sep* and *fnbB*. This study highlights the threat posed by the increasing prevalence of CO-MSSA BSIs and suggests an association with the increasing prevalence of CC45 CO-MSSA, decreasing prevalence of CC30 HO-MSSA, an ageing population and an overall decrease in virulence and resistance genes.

Earls ME, Shore AC, Brennan GI, Simbeck A, Schneider-Brachert W, Vremeră T, Dorneanu OS, Slickers P, Ehricht R, Monecke S, Coleman DC. A novel multidrug-resistant PVL-negative CC1-MRSA-IV clone emerging in Ireland and Germany likely originated in South-Eastern Europe. *Infect Genet Evol*. **69:** Apr; 117-29

Abstract

This study investigated the recent emergence of multidrug-resistant Panton-Valentine leukocidin (PVL)-negative CC1-MRSA-IV in Ireland and Germany. Ten CC1-MSSA and 139 CC1-MRSA isolates recovered in Ireland between 2004 and 2017 were investigated. These were compared to 21 German CC1-MRSA, 10 Romanian CC1-MSSA, five Romanian CC1-MRSA and two UAE CC1-MRSA, which were selected from an extensive global database, based on similar DNA microarray profiles to the Irish isolates.

All isolates subsequently underwent whole-genome sequencing, core-genome single nucleotide polymorphism (cgSNP) analysis and enhanced SCC*mec* subtyping. Two PVL-negative clades (A and B1) were identified among four main clades.

Clade A included 20 German isolates, 119 Irish isolates, and all Romanian MRSA and MSSA isolates, the latter of which differed from clade A MRSA by 47-130 cgSNPs. Eighty-six Irish clade A isolates formed a tight subclade (A1) exhibiting 0-49 pairwise cgSNPs, 80 of which harboured a 46 kb conjugative plasmid carrying both ileS2, encoding high-level mupirocin resistance, and *qacA*, encoding chlorhexidine resistance. The resistance genes *aadE*, *aphA3* and sat were detected in all clade A MRSA and the majority (8/10) of clade A MSSA isolates. None of the clade A isolates harboured any enterotoxin genes other than *seh*, which is universally present in CC1.

Clade B1 included the remaining German isolate, 17 Irish isolates and the two UAE isolates, all of which corresponded to the Western Australia MRSA-1 (WA MRSA-1) clone based on genotypic characteristics. MRSA within clades A and B1 differed by 188 cgSNPs and clade-specific SCC*mec* characteristics were identified, indicating independent acquisition of the SCC*mec* element. This study demonstrated the existence of a European PVL-negative CC1-MRSA-IV clone that is distinctly different from the well-defined PVL-negative CC1-MRSA-IV clone, WA MRSA-1. Furthermore, cgSNP analysis revealed that this newly defined clone may have originated in South-Eastern Europe, before spreading to both Ireland and Germany.

RESOURCES

Staff

During 2019 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 located 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 a number of 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 €345,000.

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