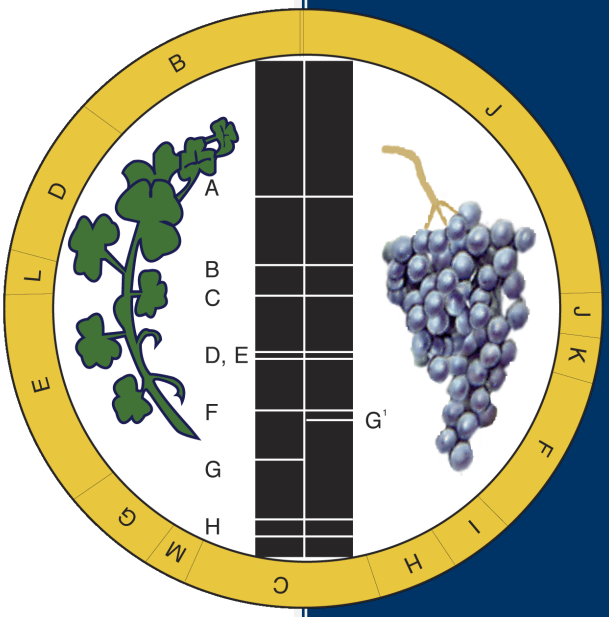
ANNUAL REPORT 2020



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 2020. Despite changes to working practices arising due to Covid, throughout 2020, the laboratory continued to deliver on its role in assisting medical professionals is the control of MRSA in hospitals and the community in Ireland.

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

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

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

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

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

Dr. Brian O'Connell
Director

Dr. Gráinne Brennan
Chief Medical Scientist

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SUMMARY

Public health impact	•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 2020, the EARS-Net project accounted for 8.2% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for 46% •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	86	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	800	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	68	Outbreak/cluster investigations (n=68) throughout Ireland
MRSA and MSSA	Confirmation of resistance against various antibiotic agents	595	Confirmation of resistance against glycopeptides, β-lactams, daptomycin and newer agents.
VRE and CoNS	Confirmation of linezolid resistance	95	Characterisation of resistance mechanism associated with increased linezolid resistance in VRE and CoNS

REFERENCE LABORATORY WORK

As the number of MRSA isolates recovered from blood stream infections reduced in 2020 (1), so too did the workload associated with these isolates accounting for 8.2% of the overall work of the laboratory. Despite this however the number of isolates submitted to the laboratory continues to increase with MSSA isolates and non-*S. aureus* isolates accounted for over 46% (Fig. 1).

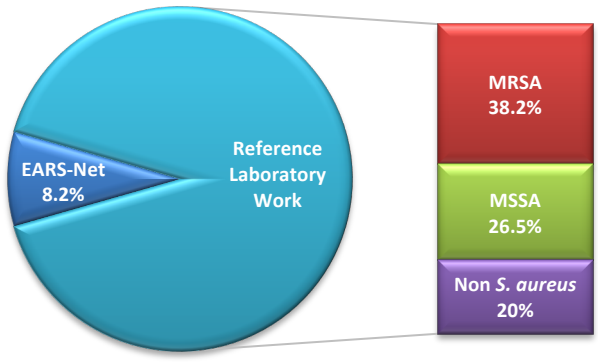


Fig 1 Workload of the NMRSARL during 2020

In recent years an increase in requests for investigations of MSSA isolates has led to a change in the services of the laboratory and 2020 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 staphylococcal isolates submitted however further molecular investigation is also performed on over half of these isolates including investigation for PVL toxin (n=587) or *spa* typing (n=548). 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.

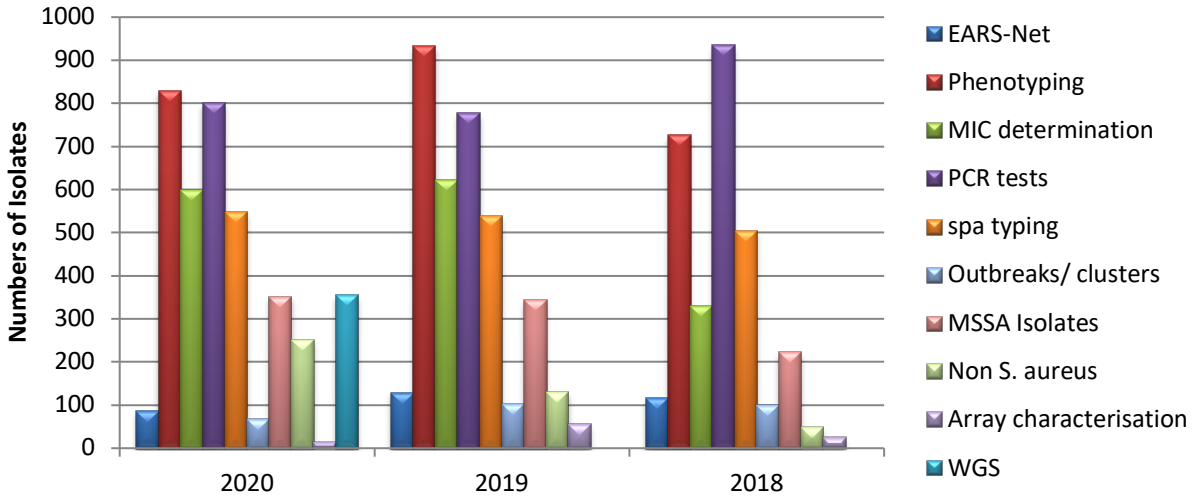


Fig 2 Distribution of workload throughout 2020

*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.

ANTIMICROBIAL RESISTANCE AMONG MRSA IN IRELAND

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

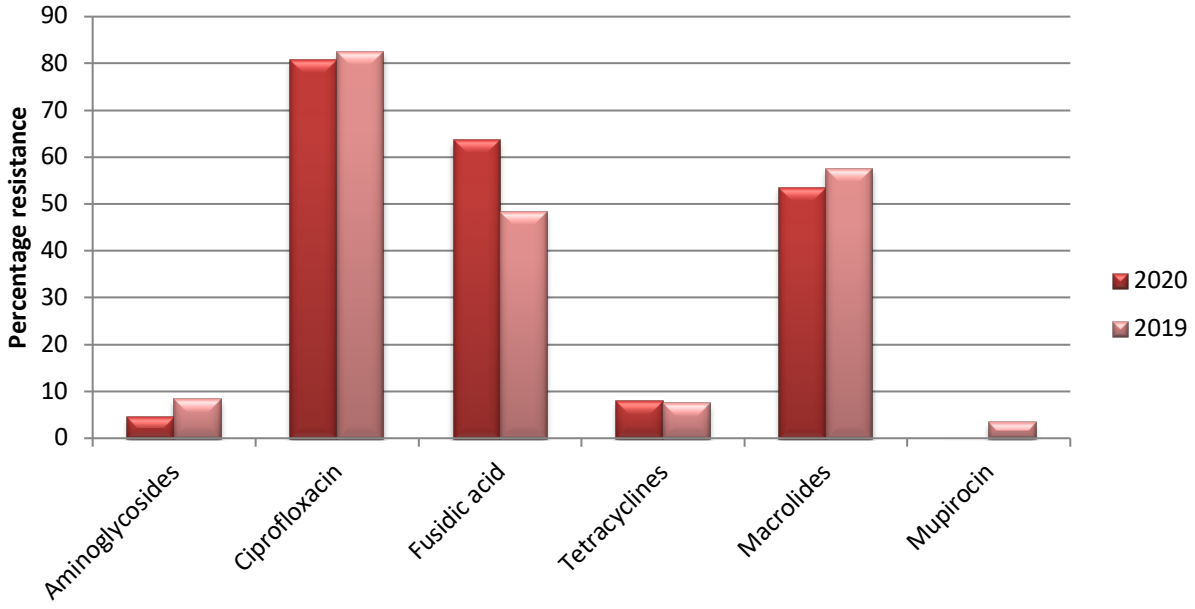


Fig 3 Resistance rates among EARS-Net isolates recovered in 2020

Antimicrobial susceptibility among MRSA recovered from non- blood stream infections

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

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

Below shows the profile of all non-BSI isolates investigated in comparison to those of BSI

isolates. In Ireland ST22-MRSA-IV is the predominant HA-MRSA accounting for 80% of MRSA investigated under the EARS-Net project and exhibits a non-multiantibiotic resistant profile. However, the non-BSI isolates recovered both in healthcare facilities and in the community, and which may also be among others, ST22-MRSA-IV, exhibit higher levels of resistance against the panel of antibiotics tested with 74% of isolates exhibiting multi-antibiotic resistance, that is, resistance to three or more different classes of antibiotics and to aminoglycosides, mupirocin, and tetracycline.

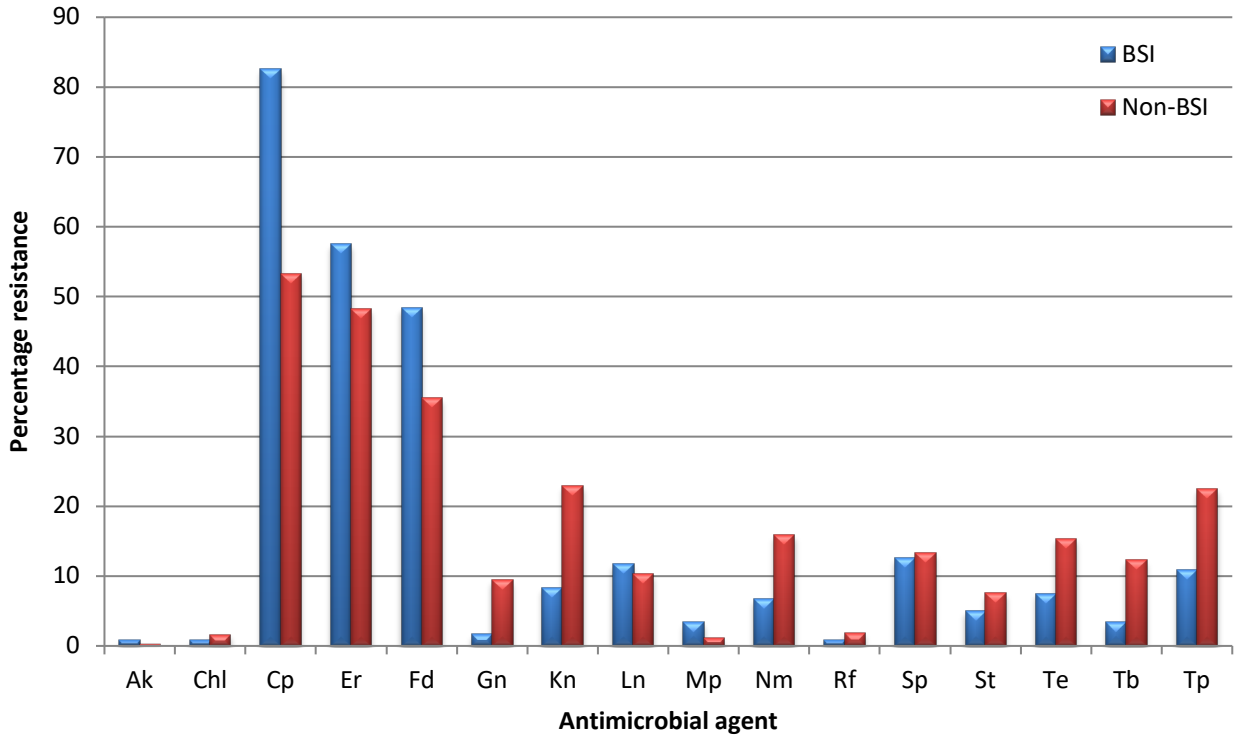


Fig 4 The percentage of blood stream MRSA isolates exhibiting resistance to each antimicrobial agent in comparison to those recovered from non-blood stream infections.

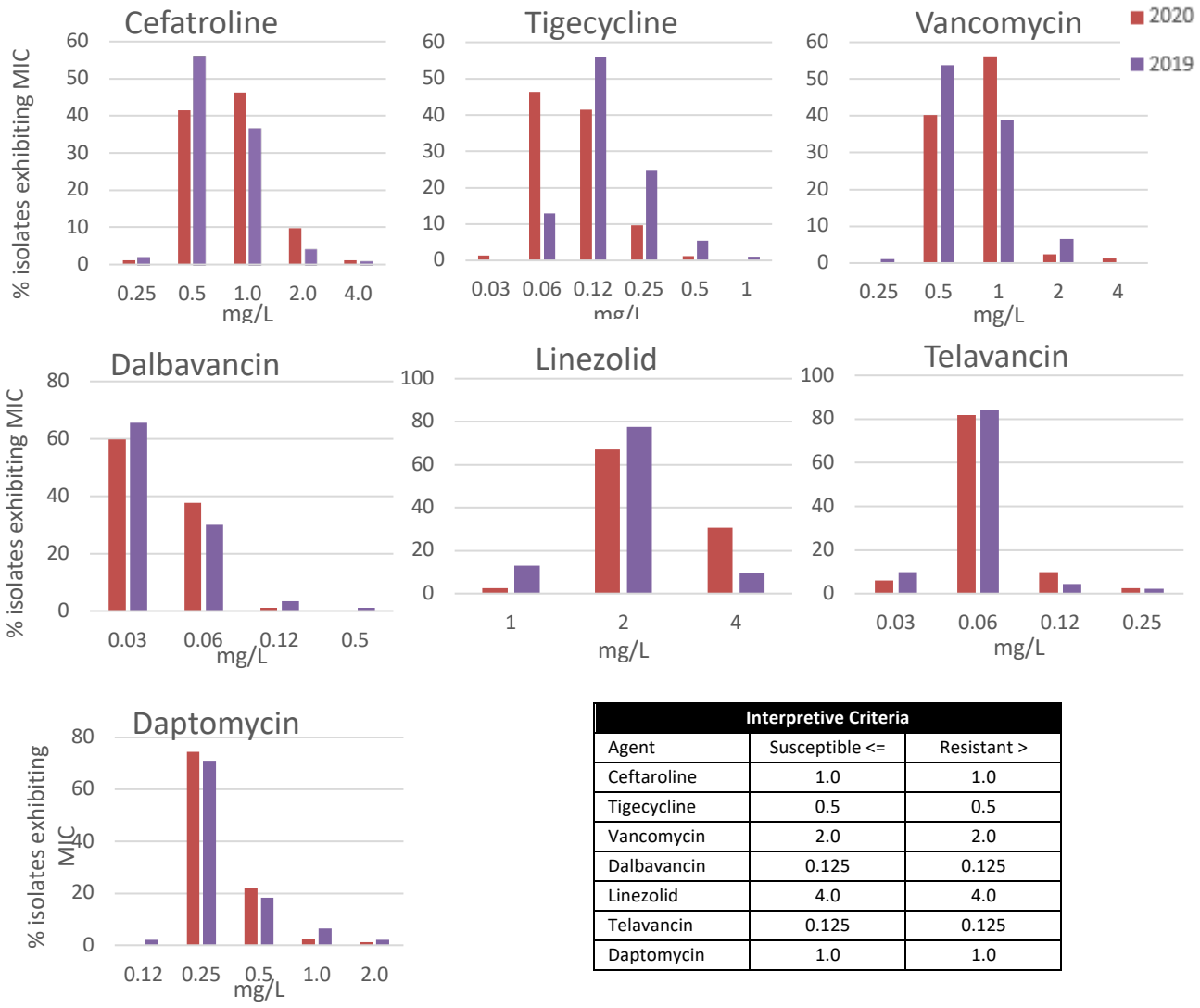
Resistance patterns determined for MRSA isolates by antibiogram- resistogram typing. Percentage for each agent includes those exhibiting resistance as determined in accordance with EUCAST or in-house developed interpretive criteria. Abbreviations: Ak; amikacin, Chl; chloramphenicol, Cp; ciprofloxacin, 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 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 2019.



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
Telavancin	0.125	0.125
Daptomycin	1.0	1.0

Linezolid resistance in Staphylococci and Enterococci

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

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

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

During 2020 there were 88 isolates investigated for these transferrable resistance genes. Among isolates investigated 86.4% (72/88) exhibited phenotypic resistance, 10.3% (9/88) were found to harbour *optrA* while 6.9% (6/88) were positive for the *poxtA* gene. One isolate harboured both *optrA* and *poxtA*. Mutational resistance associated with 23S rRNA was not investigated however recently the laboratory has introduced a test for the G2576T mutation for all future isolates.

EPIDEMIOLOGICAL TYPING OF MRSA IN IRELAND

For several 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. 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 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).

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 Southeast 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.

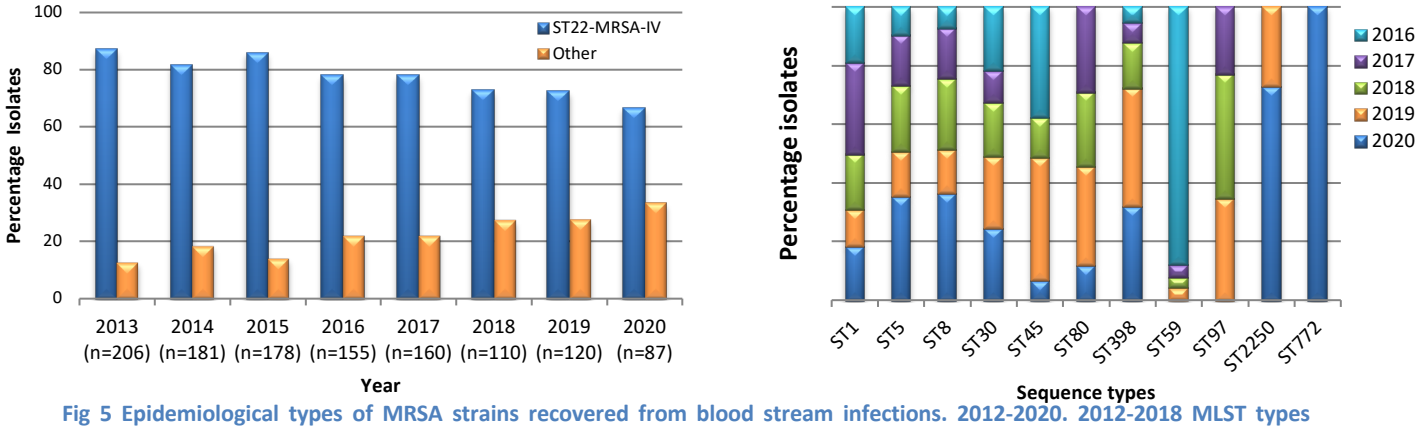


Fig 5 Epidemiological types of MRSA strains recovered from blood stream infections. 2012-2020. 2012-2018 MLST types inferred using *spa* typing and antibiogram resistogram (AR) typing. MLST assigned to isolates investigated in 2019-2020 following whole genome sequencing analysis. The total number of isolates investigated each year is shown in parentheses. Also shown is the percentage of isolates assigned to each sequence type each year.

ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND

Like Europe, ST22-MRSA-IV is the pandemic clone in Ireland and, in 2020, was associated with 66.6% of MRSA causing blood stream infections. This strain is also known as UK-EMRSA-15, Barnim Epidemic Strain, Spanish PFGE type E13 or Canadian MRSA-8 (4).

This strain has been reported in many countries and, where present, tends to be the predominate strain accounting for >50% of MRSA in Portugal, and Malta and in England it is currently associated with 85% of bacteraemia cases. The strain occurs in hospitals as well as among outpatients in the community and has also been recovered from companion animals such as horses, cats and dogs (4).

Due to the low discriminatory power of current bacterial epidemiological typing techniques such as *spa* typing when differentiating ST22-MRSA-IV in Irish hospitals, the NMRSARL utilized whole genome sequencing technology to provide detailed analysis of the ST22-MRSA-IV isolates (n=86) recovered from blood cultures during 2020.

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 2020 EARS-Net collection, there were 11 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 6).

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*).

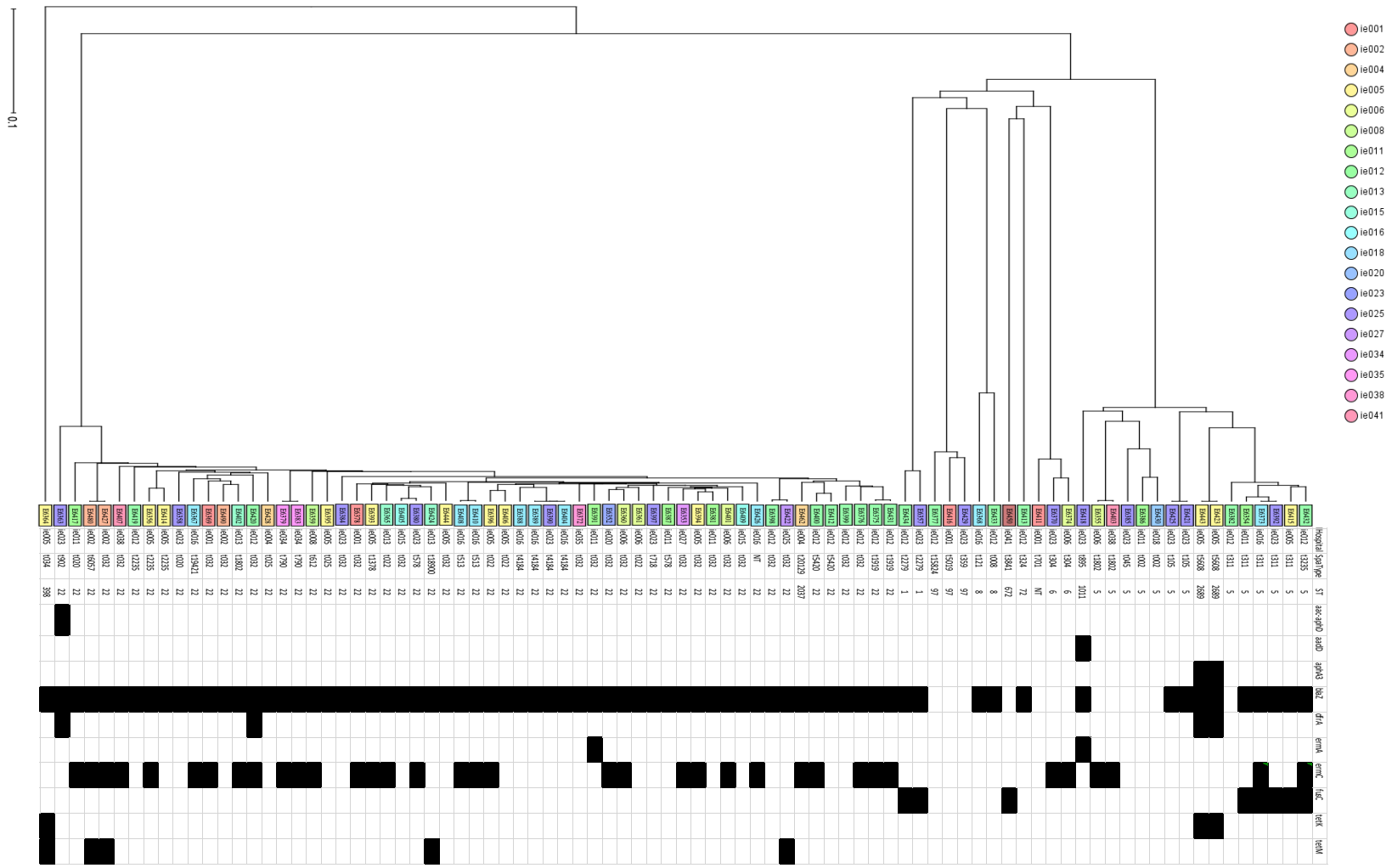


Fig 6 Phylogeny of MRSA isolates recovered from blood culture specimens during 2020. Coloured labels on tree represent different hospitals identified using their EARS-Net code. The phylogenetic tree was annotated with the distribution of selected resistance genes and where the gene was found to be present when there was >90% coverage of the gene at >30x depth of sequencing reads along with the *spa* type and MLST.

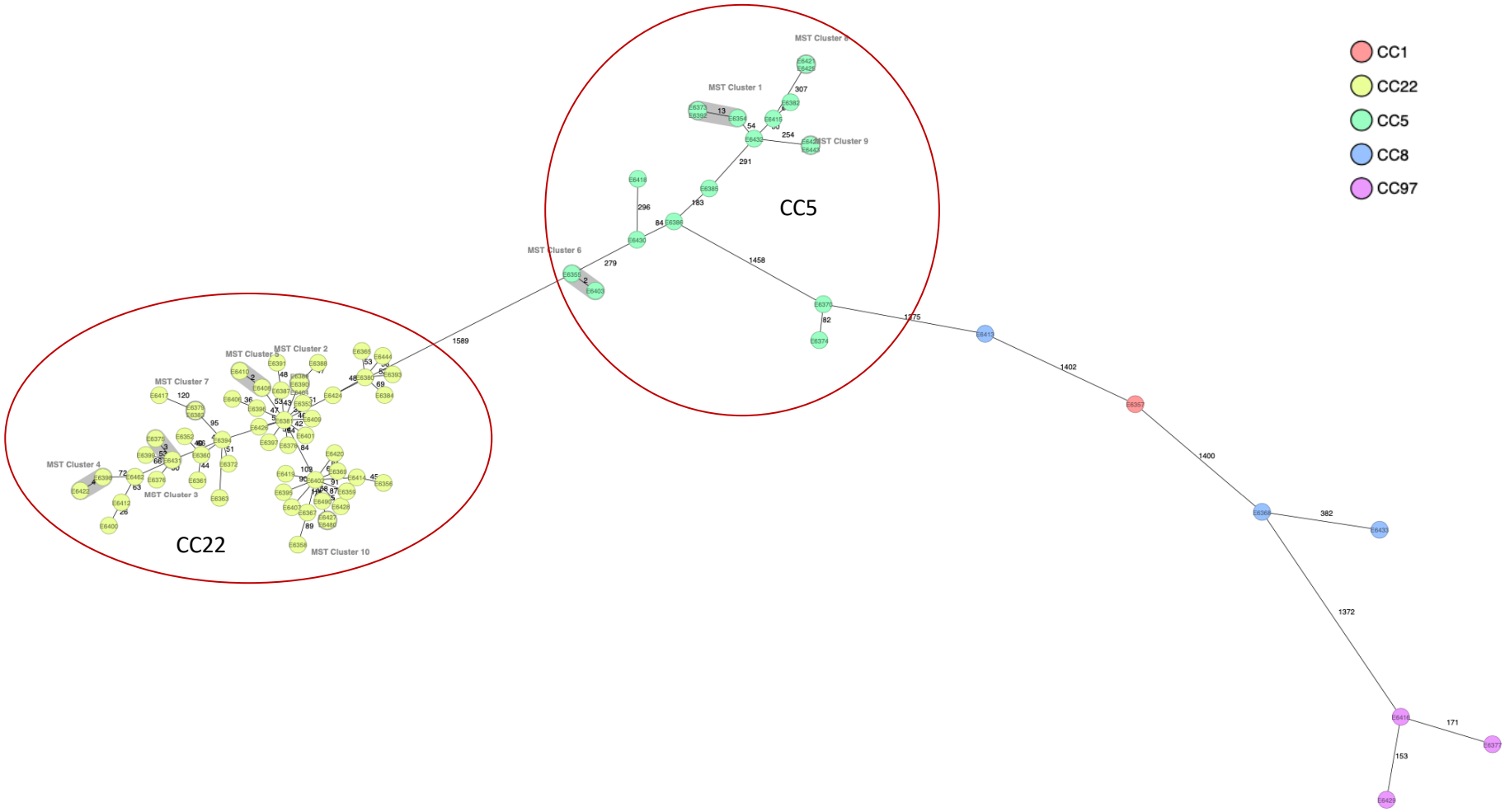


Fig 7: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all MRSA isolates recovered from blood stream infections during 2020. In each MST, MRSA isolates assigned to the same sequence type (ST) are indicated by separate colours. Closely related clusters of isolates (C1-10) (≤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.

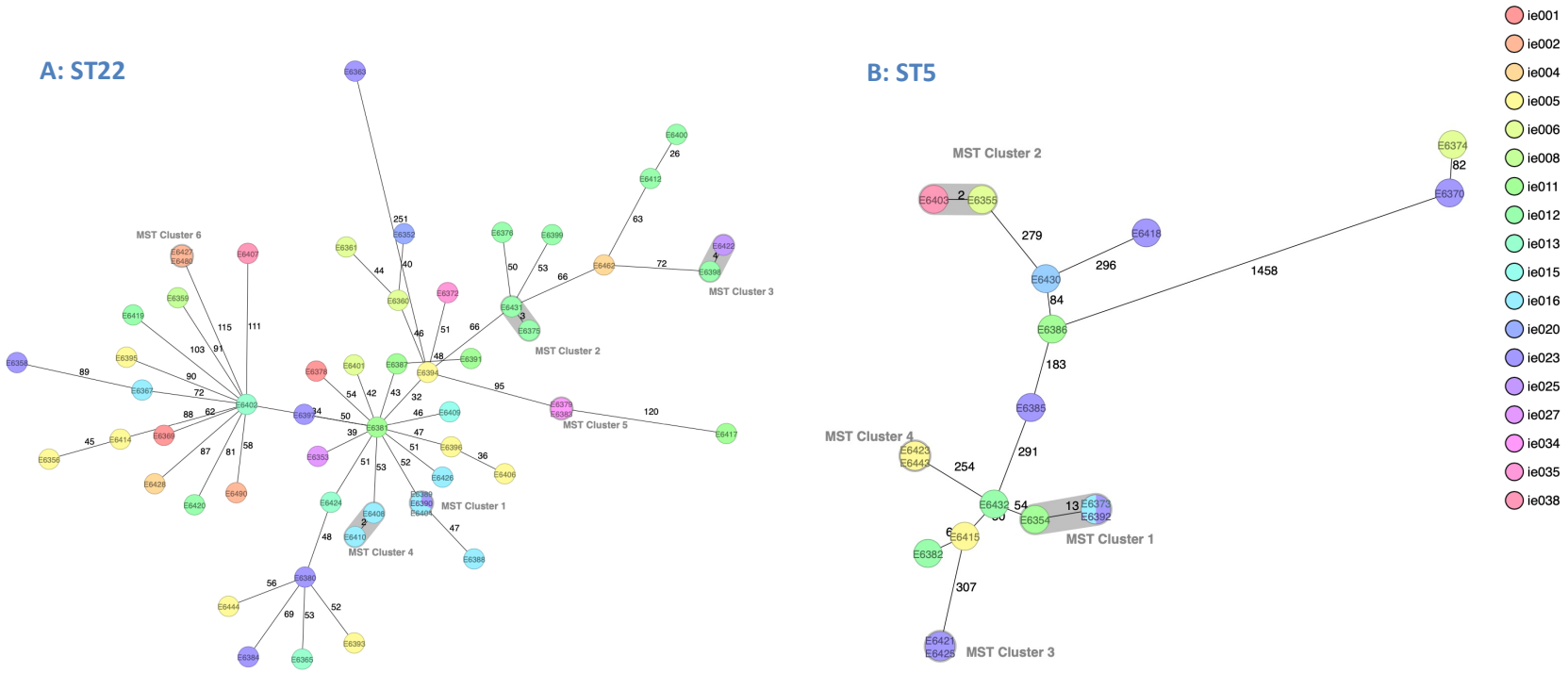


Fig 8: (A) MST constructed from the ST22 MRSA isolates. Four distinct clusters (Cluster 2, 4, 5 & 6) involved isolates recovered from the same hospitals while two other clusters (Cluster 1 & 3) involved isolates recovered from two different hospitals (Cluster 1: IE016/ IE023 and Cluster 3: IE012/ IE025). There is no further epidemiological information regarding patient transfers between these hospitals.

(B) MST constructed from ST5 MRSA isolates recovered from blood culture isolates during 2020. Four distinct clusters were recognised two of which involved isolates recovered from different hospitals (Cluster 1: ie011/ie016/ie023 and Cluster 2: ie006/ie038). There is no further epidemiological information regarding patient transfers between these hospitals.

PVL positive *S. aureus*

Throughout 2020 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 2020, 587 *S. aureus* isolates (non-BSI) were investigated for carriage of the *lukS-PV* and *lukF-PV* genes encoding for PVL. The isolates investigated included 341 MRSA and 246 MSSA.

Among the MRSA isolates 25.2% (86/341) were found to be positive while 8.5% (21/246) of MSSA isolates were also positive.

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

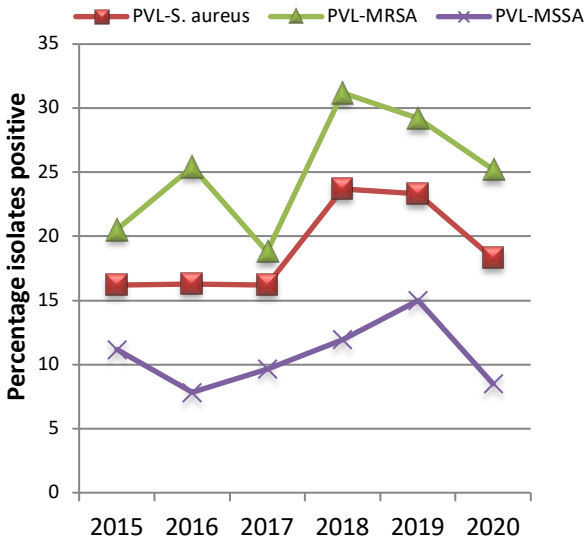


Fig 9: Frequency of PVL S. aureus

As in previous years, the distribution of epidemiological types among PVL+ *S. aureus* is limited with less diversity seen among the MRSA isolates. In 2020, 75% of the isolates were limited to only eight sequence types (Fig 10).

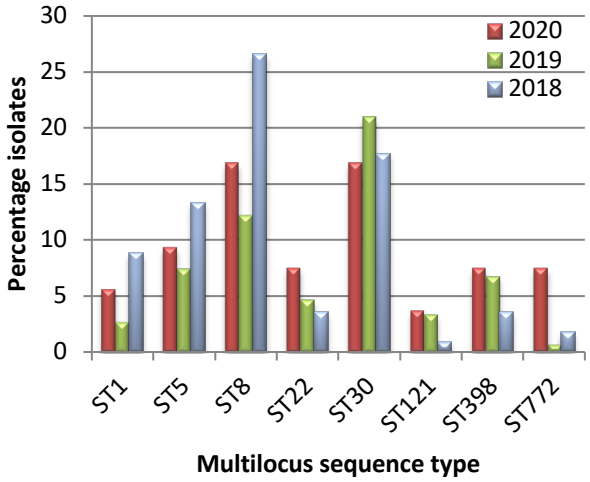


Fig 10 Distribution of sequence types among PVL-*S. aureus* isolates recovered in 2020.

Both MRSA and MSSA were found to be associated with only five STs (ST1, ST5, ST8, ST30 and ST398). There was a further increase in the frequency at which ST398-PVL *S. aureus* observed having increased from 1.6% in 2017 to 7.5% in 2020. 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 Southeast Asia.

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

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 40% of the isolates and, like previous years, included ST1, ST5, ST8 and ST30 (Fig 1).

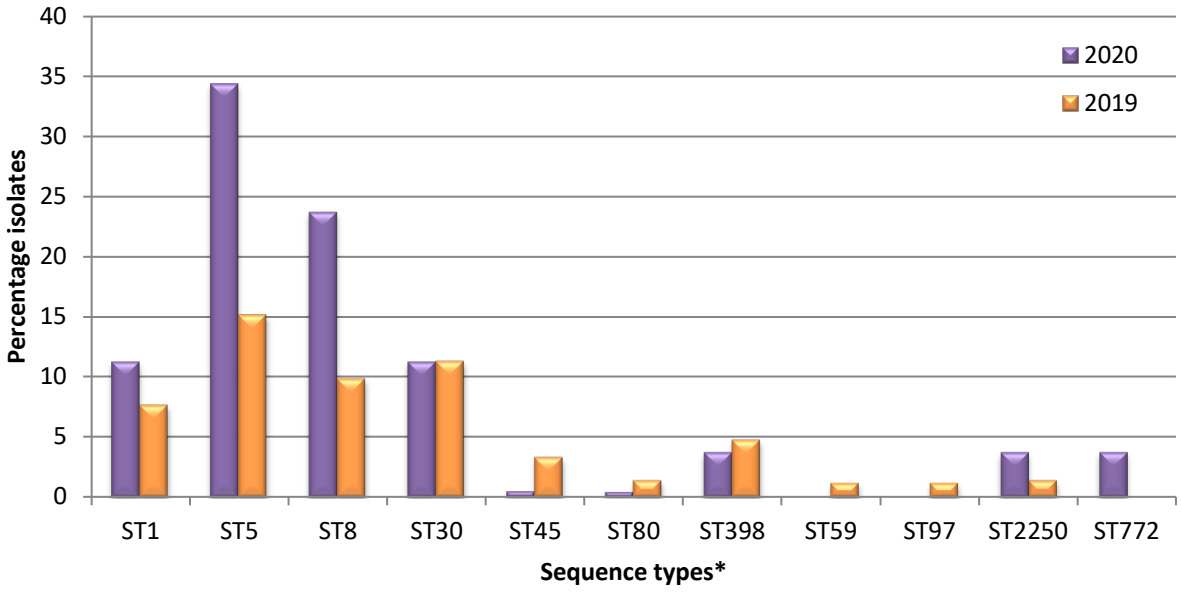


Fig 11 Most frequently recognised MLST among MRSA isolates investigated by *spa* typing during 2020

***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 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. *S. argenteus* was first described in 2009 as part of clonal complex (CC) 75 is also included in CC2250, CC1223, CC2854, and CC2198. It is usually associated with skin and soft tissue infections and has only rarely caused invasive disease. Initially *S. argenteus* was thought to be less virulent than *S. aureus*, due to the lack of the yellow pigment staphyloxantin which confers resistance against oxidative stress and neutrophil killing however *S. argenteus* has now been shown to have similar or higher mortality rates than *S. aureus*;
- human mortality rates have been reported for *S. argenteus.* causing 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
- 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.

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.

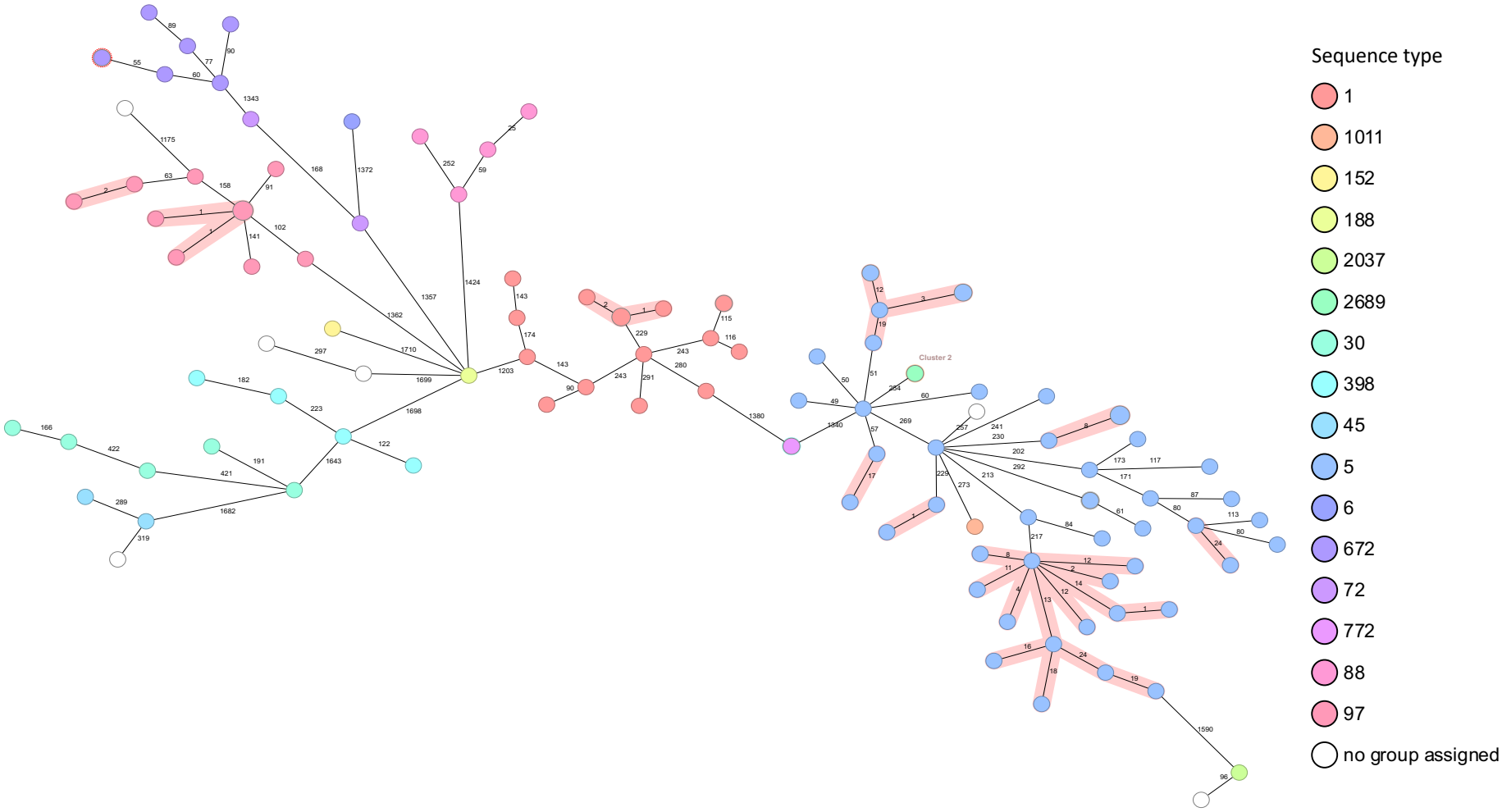


Fig 12: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all MRSA isolates associated with CA-MRSA lineages investigated by whole genome sequencing. In each MST, MRSA isolates assigned to the same sequence type (ST) are indicated by separate colours. Closely related clusters of isolates (≤24 cgMLST allelic differences) are outlined with red shadowing. The numbers on each branch indicate the numbers of cgMLST allelic differences detected between neighbouring isolates.

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 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, has only once been recovered from an animal in Ireland.

CC398-MRSA

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

In contrast however, PVL-positive ST398 has continued to increase in prevalence in Ireland. This strain is frequently reported from Southeast 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.

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;

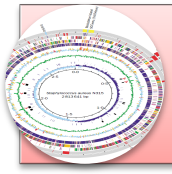
- Journal clubs
- Focus on Infection
- Antimicrobial Resistance
- Microbiology Advisory Body

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

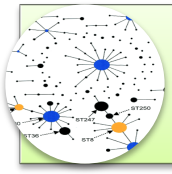
RESEARCH HIGHLIGHTS

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

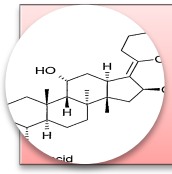
- Characterisation of the genotypes, virulence and antimicrobial resistance genes of *pvl*-positive MRSA in Ireland and MRSA in closed communities

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

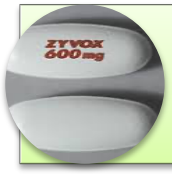
MSSA

- 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.

Egan SA *et al.,* Linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis* from hospitalized patients in Ireland: high prevalence of the MDR genes *optrA* and *poxtA* in isolates with diverse genetic backgrounds. (2020) J Antimicrob Chemother. 75; 7:1704-11.

Abstract

Objectives: To investigate the prevalence of the *optrA, poxtA* and *cfr* linezolid resistance genes in linezolid-resistant enterococci from Irish hospitals and to characterize associated plasmids.

Methods: One hundred and fifty-four linezolid-resistant isolates recovered in 14 hospitals between June 2016 and August 2019 were screened for resistance genes by PCR. All isolates harbouring resistance genes, and 20 without, underwent Illumina MiSeq WGS. Isolate relatedness was assessed using enterococcal whole-genome MLST. MinION sequencing (Oxford Nanopore) and hybrid assembly were used to resolve genetic environments/plasmids surrounding resistance genes.

Results: *optrA* and/or *poxtA* were identified in 35/154 (22.7%) isolates, the highest prevalence reported to date. Fifteen isolates with diverse STs harboured *optrA* only; one Enterococcus faecium isolate harboured *optrA* (chromosome) and *poxtA* (plasmid). Seven *Enterococcus faecalis* and one *E. faecium* harboured *optrA* on a 36 331 bp plasmid with 100% identity to the previously described *optrA*-encoding conjugative plasmid pE349. Variations around *optrA* were also observed, with *optrA* located on plasmids in five isolates and within the chromosome in three isolates. Nine E. faecium and 10 *E. faecalis* harboured *poxtA*, flanked by IS1216E, within an identical 4001 bp region on plasmids exhibiting 72.9%-100% sequence coverage to a 21 849 bp conjugative plasmid. *E. faecalis* isolates belonged to ST480, whereas *E. faecium* isolates belonged to diverse STs. Of the remaining 119 linezolid-resistant isolates without linezolid resistance genes, 20 investigated representatives all harboured the G2576T 23S RNA gene mutation associated with linezolid resistance.

Conclusions: This high prevalence of *optrA* and *poxtA* in diverse enterococcal lineages in Irish hospitals indicates significant selective pressure(s) for maintenance.

Egan SA *et al.,* Hospital outbreak of linezolid-resistant and vancomycin-resistant ST80 Enterococcus faecium harbouring an *optrA*-encoding conjugative plasmid investigated by whole-genome sequencing. (2020) J Hosp Infect., 105; 4: 726-35.

Abstract

Background

Linezolid is an antibiotic used to treat infections caused by multi-drug-resistant Gram-positive bacteria. Linezolid resistance in enterococci has been reported with increasing frequency, with a recent rise in resistance encoded by *optrA, poxtA* or *cfr*.

Aim

To investigate a hospital outbreak of linezolid- and vancomycin-resistant *Enterococcus faecium* (LVREfm) using whole-genome sequencing (WGS).

Methods

Thirty-nine VREfm from patient screening (19 isolates, 17 patients) and environmental sites (20 isolates) recovered in October 2019 were investigated. Isolates were screened using polymerase chain reaction for *optrA, poxtA* and *cfr*, and underwent Illumina MiSeq WGS. Isolate relatedness was assessed using E. faecium core genome multi-locus sequence typing (cgMLST). One LVREfm underwent MinION long-read WGS (Oxford Nanopore Technologies) and hybrid assembly with MiSeq short-read sequences to resolve an optrA-encoding plasmid.

Findings

Twenty isolates (51.3%) were LVREfm and optrA-positive, including the LVREfm from the index patient. A closely related cluster of 28 sequence type (ST) 80 isolates was identified by cgMLST, including all 20 LVREfm and eight linezolid-susceptible VREfm, with an average allelic difference of two (range 0–10), indicating an outbreak. Nineteen (95%) LVREfm harboured a 56,684-bp conjugative plasmid (pEfmO_03). The remaining LVREfm exhibited 44.1% sequence coverage to pEfmO_03. The presence of pEfmO_03 in LVREfm and the close relatedness of the outbreak cluster isolates indicated the spread of a single strain. The outbreak was terminated by enhanced infection prevention and control (IPC) and environmental cleaning measures, ceasing ward admissions and ward-dedicated staff.

Conclusion

WGS was central in investigating an outbreak of ST80 LVREfm. The rapid implementation of enhanced IPC measures terminated the outbreak.

McManus B *et al.,* Comparative Microbiological and Whole-Genome Analysis of *Staphylococcus aureus* Populations in the Oro-Nasal Cavities, Skin and Diabetic Foot Ulcers of Patients With Type 2 Diabetes Reveals a Possible Oro-Nasal Reservoir for Ulcer Infection. (2020) Front Microbiol Apr 30; 11: 748.

Abstract

Patients with type 2 diabetes are at higher risk for periodontal disease and diabetic foot ulcer infections (DFUIs), the latter of which are predominantly caused by staphylococcal bacteria. Staphylococci have also been detected in the mouth, nose and gums (the oro-nasal cavity) of patients with periodontal disease and can move between the mouth and nose.

The present study investigated if the oro-nasal cavity and/or periodontal pockets (PPs) in diseased gum tissue can provide a microbial reservoir for DFUIs. Eighteen patients with type 2 diabetes and at least three natural teeth (13 patients with ulcers and 5 patients without ulcers) underwent non-invasive microbiological sampling of PP, oro-nasal, skin and ulcer sites.

Staphylococci were recovered using selective chromogenic agar, definitively identified and subjected to DNA microarray profiling, whole-genome sequencing and core-genome multilocus sequence typing (cgMLST). *Staphylococcus aureus* and *Staphylococcus epidermidis* were recovered from both the oro-nasal and ulcer sites of 6/13 and 5/13 patients with ulcers, respectively.

Molecular typing based on the staphylococcal protein A (*spa*) gene and DNA microarray profiling indicated that for each patient investigated, *S. aureus* strains from oro-nasal and ulcer sites were identical. Comparative cgMLST confirmed that isolates from multiple anatomical sites of each individual investigated grouped into closely related, patient-distinct clusters (Clusters 1-7). Isolates belonging to the same cluster exhibited an average of 2.9 allelic differences (range 0-11). In contrast, reference genomes downloaded from GenBank selected as representatives of each sequence type identified in the present study exhibited an average of 227 allelic differences from the most closely related isolate within each cluster.

Moloney EM *et al.,* Whole-genome sequencing identifies highly related *Pseudomonas aeruginosa* strains in multiple washbasin U-bends at several locations in one hospital: evidence for trafficking of potential pathogens via wastewater pipes. (2020) J Hosp Infect. 104**;** 4: 484-91.

Abstract

Background: Hand washbasin U-bends have increasingly been associated with nosocomial outbreaks by Gram-negative bacteria, including *Pseudomonas aeruginosa* which is virtually ubiquitous in U-bends. Wastewater networks servicing U-bends are potential highways for trafficking pathogenic bacteria.

Aim: To use *P. aeruginosa* to investigate trafficking of bacteria between hospital washbasin U-bends.

Methods: Twenty-five washbasin U-bends in five locations in Dublin Dental University Hospital (DDUH) were investigated for trafficking *of P. aeruginosa*: 10 in Clinic 2 (C2), 10 in the Accident & Emergency Department (A&E) and five in three other locations. In addition, washbasin tap samples (N=80) and mains and tap water samples (N=72) were cultured for P. aeruginosa. Selected *P. aeruginosa* isolates recovered over 29 months underwent whole-genome sequencing, and relatedness was interpreted using whole-genome multi-locus sequence typing and pairwise single nucleotide polymorphism (SNP) analysis.

Findings: *P. aeruginosa* was recovered from all U-bends but not from taps or water. Eighty-three U-bend isolates yielded 10 sequence types (STs), with ST560 and ST179 from A&E, C2 and two other locations predominating (70%). ST560 was also recovered from a common downstream pipe. Isolates within ST560 and ST179 were highly related regardless of source. ST560 was divided into Cluster I (N=25) and Cluster II (N=2) with average allelic differences and SNPs of three and zero, and two and five, respectively. The 31 ST179 isolates exhibited an average allelic difference and SNPs of three and 12, respectively.

Conclusion: Highly related *P. aeruginosa* strains were identified in multiple U-bends in several DDUH locations, indicating trafficking via the wastewater network.

Abbot Y *et al.,* Toxigenic *Corynebacterium ulcerans* associated with upper respiratory infections in cats and dogs (2020). J Small Anim Pract 61; 9: 554-560.

Abstract

Objectives: To describe infection in companion animals with the zoonotic pathogen *Corynebacterium ulcerans* and to determine its prevalence in clinically-affected and healthy animals.

Materials and methods: The clinical presentation and treatment of three cases of *C. ulcerans* infection is described. Two studies to determine *C. ulcerans* prevalence rates were undertaken: (a) a prospective study of nasal samples from healthy animals, 479 dogs and 72 cats; (b) a retrospective analysis of records of nasal samples collected over a 10-year period from 189 dogs and 64 cats affected by respiratory signs.

Results: Toxigenic *C. ulcerans* was isolated from four cats with nasal discharge while concurrent *C. ulcerans* and *mecC* methicillin-resistant *S. aureus* infection was detected in a dog suffering from chronic nasal discharge. Clinical features were not distinctive and all cases recovered following antimicrobial treatment. Multilocus sequence typing supported a common source for isolates from the shelter cats. Carriage rates of *C. ulcerans* in healthy animals were 0.42% (2/479) in dogs and 0.00% (0/72) in cats whereas in animals with signs of upper respiratory tract infection prevalence rates were 0.53% (1/189) in dogs and 6.25% (4/64) in cats.

Clinical significance: Clinicians should be aware that dogs and cats can be infected with (or carriers of) toxigenic *C. ulcerans* Considering the potential zoonotic risk, assistance from medical and public health colleagues should be sought in confirmed cases.

RESOURCES

Staff

During 2020 the staff working in the NMRSARL were:

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

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

Administration

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

Facilities

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

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

Finance

The budget allocated to the NMRSARL for the year to cover both pay and non-pay elements amounted to €316,000.

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