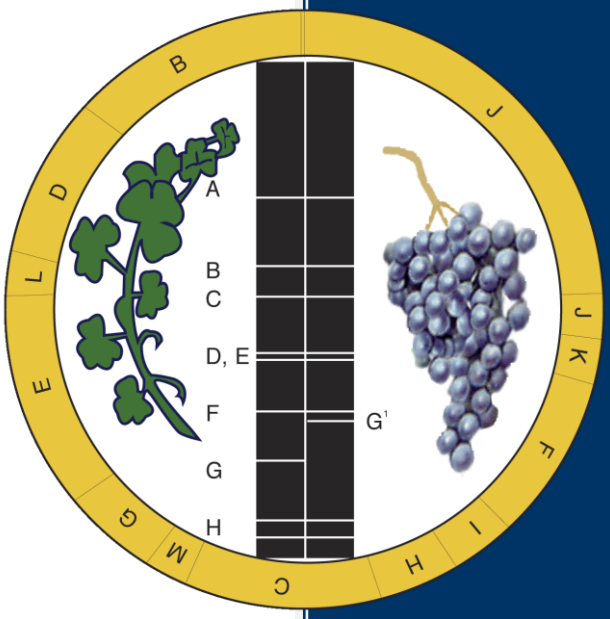
ANNUAL REPORT 2016



National Meticillin-Resistant *Staphylococcus aureus* Reference Laboratory

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Contents

INTRODUCTION

This annual report details the work of the National MRSA Reference Laboratory in 2016. Throughout 2016, the laboratory continued to deliver on its role in assisting medical professionals is the control of MRSA in hospitals and the community in Ireland.

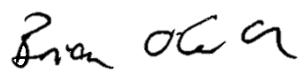
In addition to the routine work of the laboratory which includes molecular typing for epidemiological investigation and the detection of important virulence factors, service developments during the year included:

- the introduction 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;
- the staff of the laboratory continuied to provide education and training to doctors, nurses and scientists and contribute to MRSA research by completing/collaborating in numerous publications.

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

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

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



Dr. Brian O'Connell
Director

Dr. Gráinne Brennan
Chief Medical Scientist

SUMMARY

Public health impact	•The surveillance and identification of potential outbreaks of MRSA, MSSA and CoNS •The laboratory also monitors the incidence of *pvl* carrying strains of *S. aureus* and the strains associated with healthcare infections
New service developments	•Phenotypic and genotypic methods remain under constant review to take advantage of any newly developed methodologies; •Investigation of linezolid resistance among enterococci and CoNS
Activity	•Overall isolate workload increased by 5.3% based on 2015 activity with an increase of 7.9% among referral work coupled with a continuing decline in EARS-Net workload
Research and development	•The laboratory continues to participate in numerous projects and is collaborating with the Dublin Dental University Hospital, Trinity College to evaluate the potential of whole genome sequencing for routine use
Education and training	•The laboratory continues to offer training to biomedical science students and postgraduate students in Trinity College Dublin and Dublin Institute of Technology •The Chief Medical Scientist completed a PhD investigating emerging strains of MRSA in the community, among livestock and in healthcare facilities in Ireland
Future developments	•As technology expands into whole genome sequencing, this technology will replace a number of the current assays and produce definitive data on the similarities and differences between organisms

ROLE OF THE LABORATORY

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

SERVICES

The NMRSARL provides the following services:

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

ISOLATES

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

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

PUBLIC HEALTH IMPACT

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

Organism	Activity	Number of isolates	Outcome
MRSA blood culture isolates	Surveillance	150	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	471	Surveillance, recognition, investigation and management of PVL *S. aureus* in Ireland
MRSA & MSSA	Surveillance analysis and identification of trends	923	Data submitted to HPSC and published in the quarterly report on the surveillance of MRSA bacteraemias in Ireland
ST1-t127-MRSA-IV	Surveillance	39	Investigation of isolates recovered from community and healthcare sources between 2013 and 2016 in order to investigate the isolate relationships and the extent of their spread.
Mupirocin resistant t127-MRSA-IV	Surveillance	16	Ongoing surveillance of strain spreading within one hospital
MRSA and MSSA	Surveillance	247	Outbreak (n=29) investigations throughout Ireland
MRSA and MSSA	Confirmation of resistance against various antibiotic agents	381	Confirmation of resistance against glycopeptides, β-lactams, daptomycin and newer agents.
VRE and CoNS	Confirmation of linezolid resistance	45	Characterisation of resistance mechanism associated with increased linezolid resistance in VRE and CoNS
MSSA & MRSA	Characterisation of *S. aureus* recovered from closed community	83	Determine prevalence of *S. aureus* among closed communities in Ireland. Isolates were recovered from prisoners in a prison in the midlands.
LA-MRSA	Surveillance	5	Characterisation of MRSA strains recovered from humans but with known association to livestock. There is increasing concern about staphylococcal transmission to humans from animal associated lineages.

REFERENCE LABORATORY WORK

During 2016, work under the EARS-Net project accounted for 17% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for 36% (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 2016 saw a further expansion of service due to increasing reports 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 isolates submitted however further molecular investigation is performed on over half of the isolates including investigation for PVL toxin (n=445) or *spa* typing (n=378). This change is primarily due to the changing epidemiology of MRSA circulating in Ireland and the limited information that can be obtained from phenotypic investigation of these emerging strains.

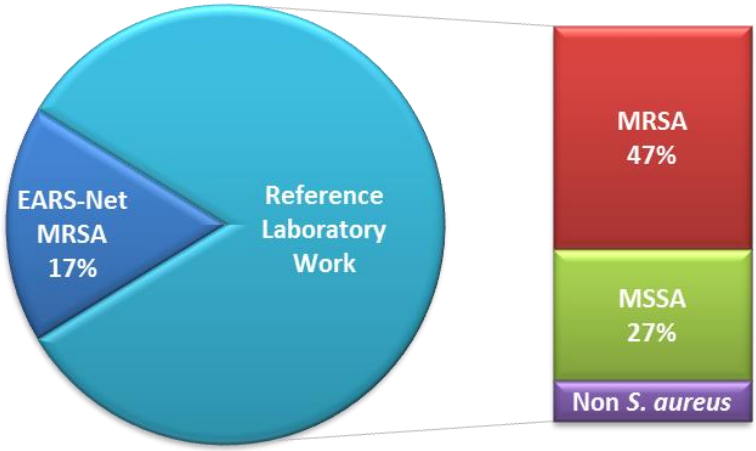


Figure 1 Workload of the NMRSARL during 2016

Borderline oxacillin resistant *S. aureus* isolates

Over the past year there have been an increasing number isolates reported as BORSA or sent as BORSA asking for confirmation by the source laboratory.

Typically, the BORSA phenotype results from an excess in the production of β-lactamase. These strains are neither heteroresistant nor multiresistant. They produce large amounts of normal staphylococcal β-lactamase which partially hydrolyzes oxacillin but become fully susceptible to oxacillin in the presence of β-lactamase inhibitors. However, the borderline phenotype has also been attributed to other mechanisms, i.e., the production of an inducible, plasmid-mediated methicillinase or different modifications in the PBP genes due to spontaneous amino acid substitutions in the transpeptidase domain.

Linezolid resistance in Staphylococci and Enterococci

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

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

As reported by the HPSC, since 2010, over 95% of notified invasive enterococcal isolates nationally have had accompanying linezolid antimicrobial susceptibility results reported to EARS-Net. Over the six-year period from 2010 to 2015, 34 of 2340 (1.4%) *E. faecium* and 16 of 1746 (1%) *E. faecalis* bloodstream isolates were reported non-susceptible to linezolid by 18 and 19 microbiology laboratories, respectively. In addition, *cfr* has been recognized in several *S. aureus* isolate and has also been associated with several linezolid resistant *Staphylococcus epidermidis* outbreak in hospitals throughout Ireland.

While the numbers investigated to date remain low, *optrA* has been detected in 9.3% (7/75) of isolates with 42% of these isolates *E. faecalis* and the remaining 58% (4/7) *E. faecium.*

PVL positive *S. aureus*

A significant number of users are requesting PVL toxin testing when submitting isolates. In 2016, 445 *S. aureus* isolates were tested for carriage of the *lukS-PV* and *lukF-PV* genes encoding for PVL comprising of 234 MRSA and 211 MSSA.

Despite a slight increase of PVL-positive MRSA (21% in 2015 to 25.6% in 2016), overall 16.4% of isolates were positive which is consistent with previous years.

The previous predominant PVL-positive MRSA t008 strain however was replaced with *spa* type t019, which is associated with ST30 and the slight increase reported in 2015 has further increased to 30% of PVL- positive isolates during 2016.

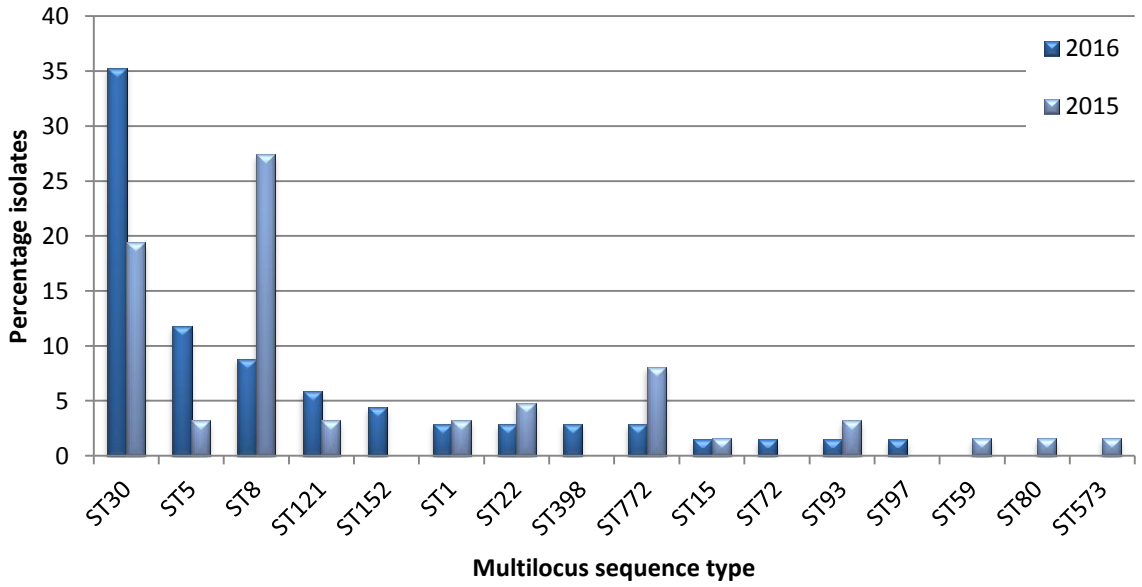


Figure 2 Multilocus sequence types of PVL positive S. aureus isolates recovered throughout 2016

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. While the most prevalent MRSA strain circulating in Irish hospitals exhibits a non-multiantibiotic resistant susceptibility profile, emerging community associated strains carry multiple virulence and resistance genes is a concern.

During 2016, with the exception of fusidic acid and ciprofloxacin, the prevalence of resistance among isolates recovered from BSI remained consistent to previous years.

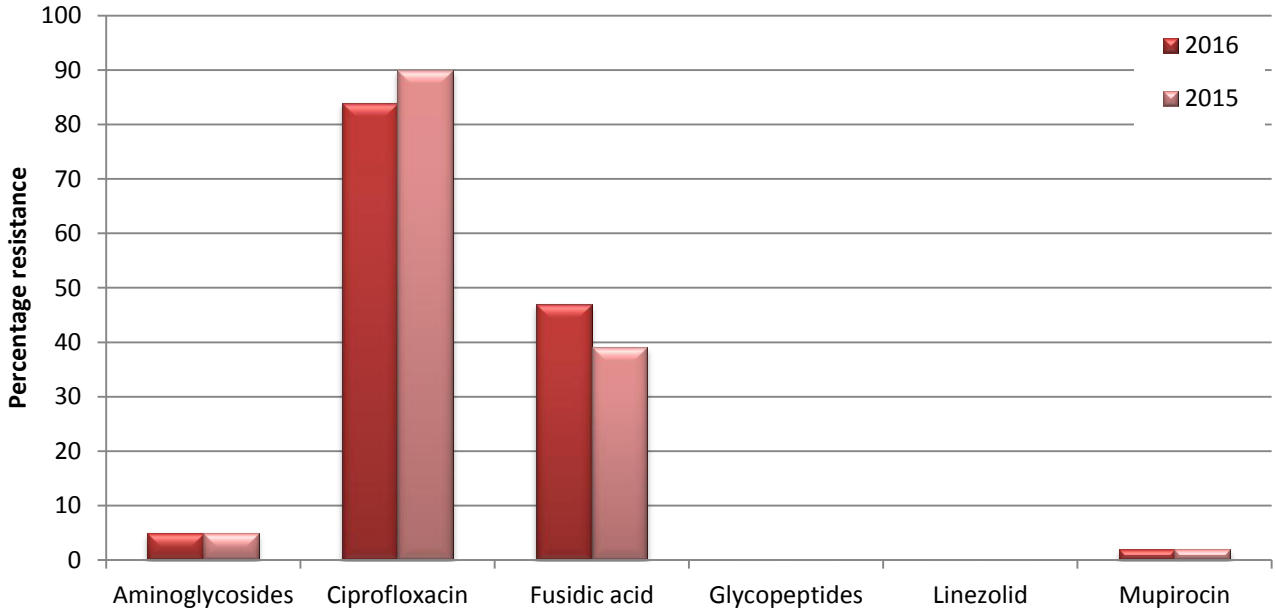


Figure 3 Resistance rates among EARS-Net isolates recovered in 2016

The increased prevalence of ciprofloxacin resistance may be associated with a slight increase in the number of ST22-MRSA-IV recovered during the year. However the increase in resistance to fusidic acid is a trend which has been observed over a number of years having increased from 27% in 2006 to the current level of 47% and is a worrying concern since fusidic acid (FA) remains a clinically useful antimicrobial for difficult to treat skin and soft tissue infections. Among the FA-resistant isolates, 82.5% are ST22-MRSA-IV and resistance among these isolates is predominantly associated with mutations in the *fusA* gene. Resistance among the remaining isolates is associated with the *fusC* gene carried on the SCC*mec* element.

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 healthcare providers expect CA-MRSA.

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 resistance to ampicillin, ciprofloxacin, erythromycin and cadmium acetate. 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 82.34% of isolates exhibiting multi-antibiotic resistance, that is, resistance to three or more different classes of antibiotics and in particular to aminoglycosides, mupirocin and tetracycline.

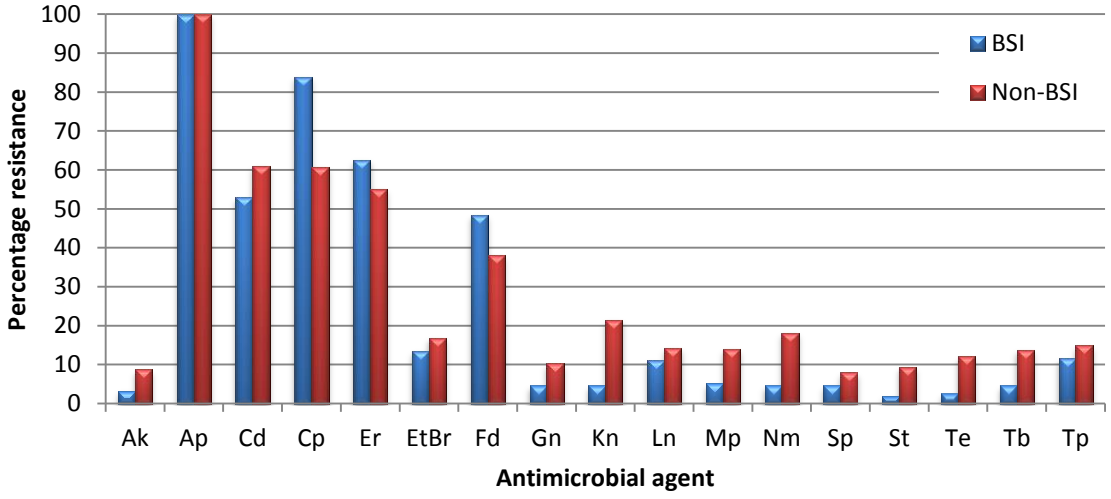


Figure 4 The percentage of blood stream isolates exhibiting resistance to each antimicrobial agent in comparison to those recovered from non-blood stream infections.

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

ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND

Like Europe, ST22-MRSA-IV is the pandemic clone in Ireland and, in 2016, was associated with 78.7% of MRSA causing blood stream infection. This strain is known also known as UK-EMRSA-15, Barnim Epidemic Strain, Spanish PFGE type E13, or Canadian MRSA-8 (2)

It 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 (2).

Common resistance patterns exhibited by the ST22-MRSA-IV strain include resistance to β-lactamase, fusidic acid, ciprofloxacin, and erythromycin. Variable virulence markers in ST22-MRSA-IV are *sec* and *sel* as well as the IEC genes encoded by lysogenic β-haemolysin-converting phages (*sak, chp, scn*) (2).

In Ireland in 2016 the most frequently occurring *spa* type among the ST22 isolates, t032 continued to be the most predominant strain however other ST22 associated *spa* types included t022, t515, t223, t2945 and t020 were also recognised.

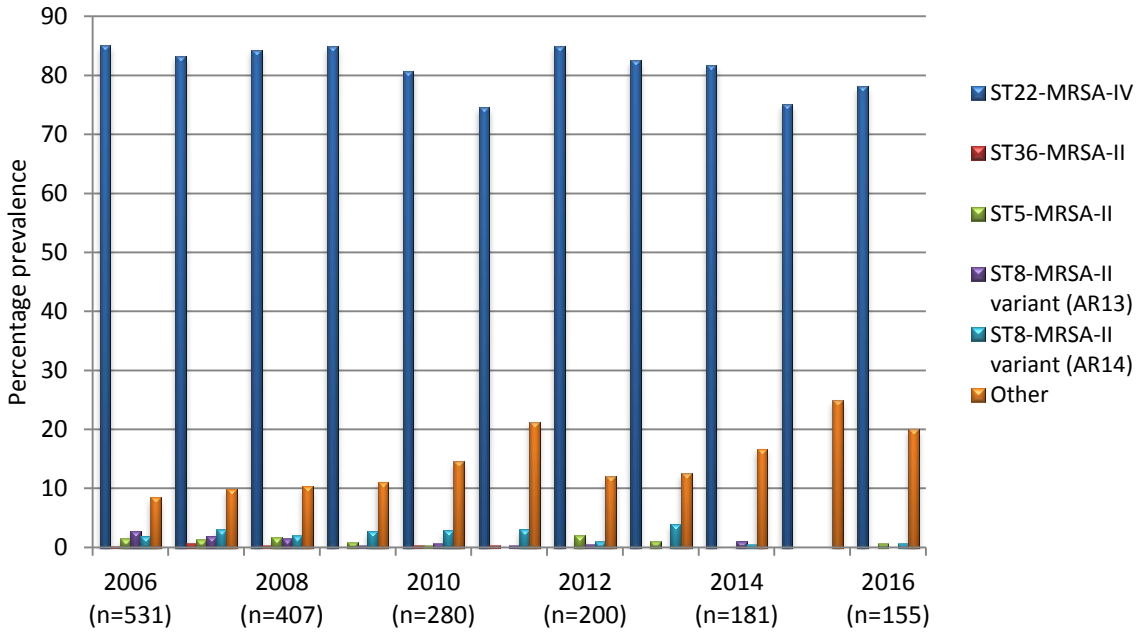


Figure 5 Epidemiological types of MRSA strains recovered from blood stream infections using antibiogram resistogram (AR) typing during 2016

MOLECULAR EPIDEMIOLOGICAL TYPING OF MRSA

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

spa typing involves sequencing of the Staphylococcal protein A gene (*spa)* to recognise mutations or repeat insertion/deletion events that can cause changes in the polymorphic X region of the *spa* gene. It has become a well-established discriminatory method for outbreak investigations but has also been shown to be useful for long-term epidemiological studies. The availability of MLST data associated with *spa* types on an online database facilitates comparison of Irish isolates with isolates from all other countries. Based upon repeating patterns (BURP) analysis clusters *spa* types together based on the repeat succession pattern of *spa* types (3) .

Using the inferred MLST data available from the *spa* typing online database the most frequently recognised MLST types accounted for 40% of the isolates and, similar to 2016 included ST1, ST5, ST8, ST30, ST45 (Fig 6). While ST1 continued to predominate, a significant increase was also detected among isolates associated with ST30. *spa* type t127 continued to be the most frequently occurring. This strain has been associated with a prolonged outbreak throughout 2014 and increased awareness of the phenotypic characteristics of the strain in other hospitals has led to an increase in the number of isolates receipted in the NMRSARL.

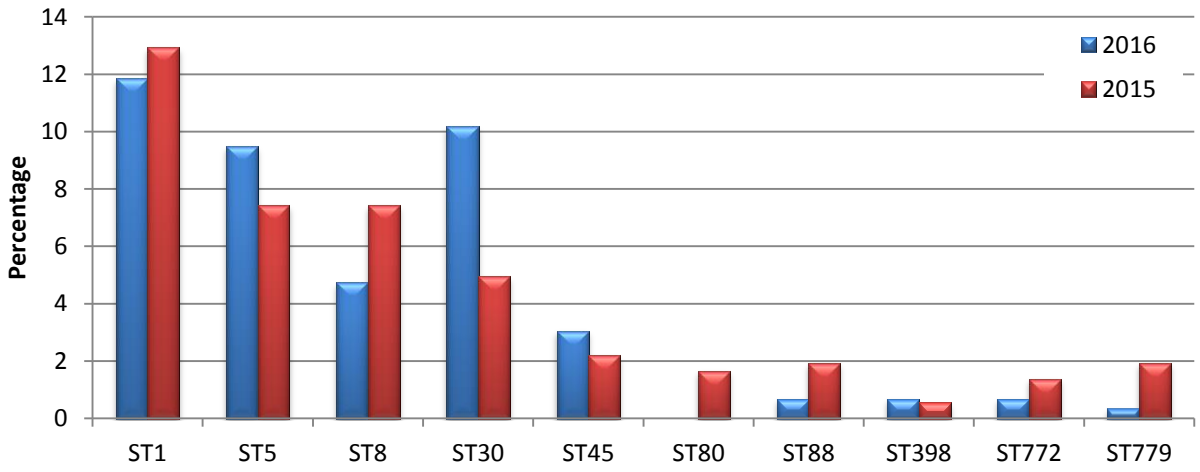


Figure 6 Most frequently recognised MLST among MRSA isolates investigated by spa typing during

THE USE OF EMERGING TECHNOLOGY IN THE NMRSARL

As previously reported in Ireland, over time, a strain displacement has occurred resulting in the predominant ST22-MRSA-IV. 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.

In order to fully characterise the emerging strains in Ireland the NMRSARL is currently utilising sequence based technology to gather information on strains circulating in hospitals and in the communities in Ireland.

DNA MICROARRAY ANALYSIS

DNA microarray profiling examines 336 *S. aureus* genes and alleles including species-specific, antimicrobial resistance and virulence associated genes potentially improving discrimination of MRSA isolates in outbreaks. The workflow is described below and isolates are selected for investigation based on initial phenotypic and genotypic analysis.

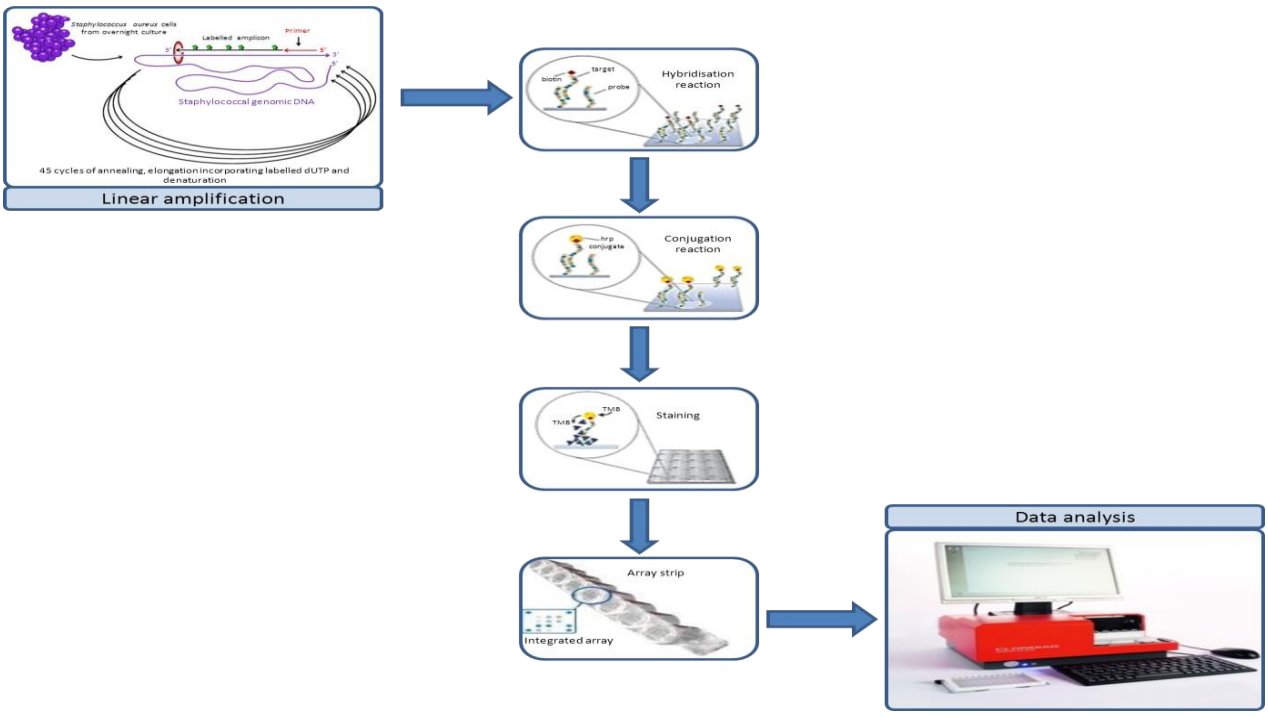


Figure 7 Schematic diagram illustrating the DNA microarray analysis process involving linear amplification of the genomic staphylococcal DNA using a single primer for all 336 gene targets and biotin labelling. The biotin labelled amplicons are hybridised to the array chips (B) followed by a conjugation reaction (C) with horseradish peroxidase conjugation (HRP-conjugate) with the final precipitation step with the tetramethylbenzidine (TMB) substrate (D). Each well on the 8-well array microarray strip contains a single DNA chip (E) and these images are analysed using the Arraymate reader, which records an image of each DNA microarray chip and the raw data is analysed and interpreted using IconoClust software (F).

WHOLE GENOME SEQUENCING

Whole-genome sequencing (WGS) is the process of determining the complete DNA sequence of a bacterial genome at a single time and, in recent years, has revolutionised tracking the spread of MRSA in both outbreak and long-term epidemiological investigations. The experience NMRSARL has with this emerging technology is limited to several collaborations with colleagues in the DDUH in determining the potential WGS has in Irish healthcare facilities.

In 2016, one such study involved WGS of ST1-t127-MRSA-IV isolates recovered from community and healthcare sources between 2013 and 2016 in order to investigate the isolate relationships and the extent of their spread. These community associated MRSA strains were selected for study due to their association with a prolonged hospital outbreak. Furthermore, this strain has increased in prevalence in recent years from <1% in 2010 to 11% of all those investigated by *spa* typing in 2016 (4).

Core-genome MLST and Single nucleotide variant (SNV) analyses revealed the recent emergence and extensive spread of two closely related strains and multiple sporadic strains of ST1-MRSA-IV-t127/t922. Within the isolates recovered from an outbreak in a large Dublin hospital, isolates of this clone were predominantly multi drug resistant and frequently high-level mupirocin resistant (Hi-MupR), the latter of which can negatively affect efforts to eradicate carriage in colonized individuals. Worryingly, the MDR CA-MRSA clone investigated in this study was detected in 17 Irish hospitals, four other Healthcare facilities and from 11 people in the community, over the last three years (2013–2016). The extensive spread of this CA-MRSA clone within and between the Irish community and hospitals/healthcare facilities highlights the potential of CA-MRSA transmission routes into hospitals (4).

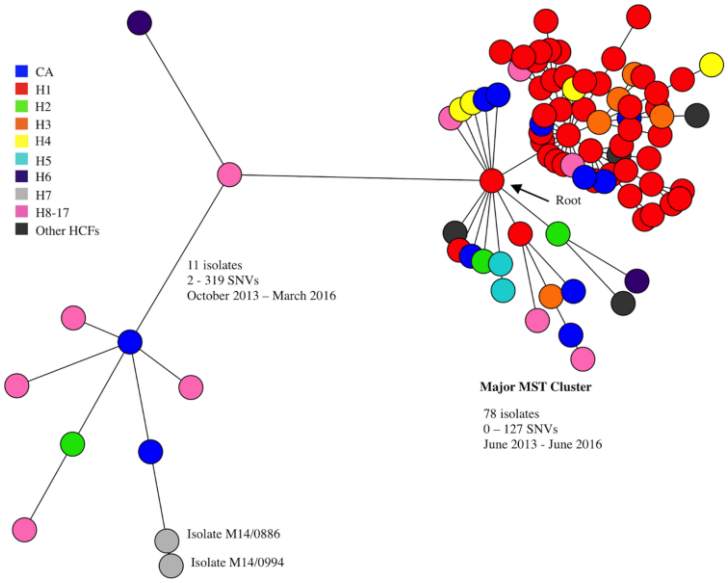


Figure 8 The pairwise single nucleotide variation (SNV) range between isolates inside and outside of the major minimum spanning tree (MST) cluster and their recovery time frame are indicated (4)**.**

Whole genome sequencing is emerging as a 'gold- standard' for the differentiation of bacterial isolates in outbreak investigations. It is anticipated that the NMRSARL will introduce WGS analysis of selected isolates in the coming 12 months. Initially it will be limited to outbreak investigations where other methods fail to adequately differentiate isolates.

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 facilitated two post graduate students undertaking projects as part of Masters of Science which involved investigating the prevalence of *S. aureus* among inmates in an Irish prison and also the characterisation of the isolates recovered from these inmates.

CONTINUOUS PROFESSIONAL DEVELOPMENT

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

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

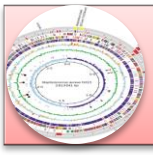
NMRSARL staff also ensured mandatory training requirements were met in areas such as;

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

One member of staff completed her MSc in Clinical Laboratory Science through DIT, Kevin Street while another member of staff completed her research PhD on the Characterisation of sporadically occurring MRSA in Ireland through the Dublin Dental Hospital, TCD.

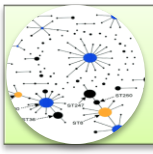
RESEARCH HIGHLIGHTS

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



Whole genome sequencing

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



Emerging MRSA strains

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

CA-MRSA

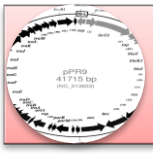
- Characterisation of the genotypes, virulence and antimicrobial resistance genes of *pvl*-positive MRSA in Ireland and MRSA in closed communities

LA-MRSA

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

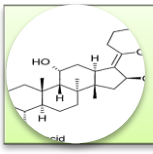
MSSA

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



Mupirocin resitance

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



Fusidic acid resistance

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

Linezolid resistance

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

PUBLICATIONS

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

First Report of *cfr*-Carrying Plasmids in the Pandemic Sequence Type 22 Methicillin-Resistant *Staphylococcus aureus* Staphylococcal Cassette Chromosome mec Type IV Clone.

Shore AC, Lazaris A, Kinnevey PM, Brennan OM, Brennan GI, O'Connell B, Feßler AT, Schwarz S, Coleman DC.

Antimicrob Agents Chemother. 2016 Apr 22; 60(5):3007-15.

Abstract

Linezolid is often the drug of last resort for serious methicillin-resistant Staphylococcus aureus (MRSA) infections. Linezolid resistance is mediated by mutations in 23S rRNA and genes for ribosomal proteins; cfr, encoding phenicol, lincosamide, oxazolidinone, pleuromutilin, and streptogramin A (PhLOPSA) resistance; its homologue *cfr*(B); or *optrA*, conferring oxazolidinone and phenicol resistance. Linezolid resistance is rare in S. aureus, and cfr is even rarer. This study investigated the clonality and linezolid resistance mechanisms of two MRSA isolates from patients in separate Irish hospitals. Isolates were subjected to *cfr* PCR, PhLOPSA susceptibility testing, 23S rRNA PCR and sequencing, DNA microarray profiling, spa typing, pulsed-field gel electrophoresis (PFGE), plasmid curing, and conjugative transfer. Whole-genome sequencing was used for single-nucleotide variant (SNV) analysis, multilocus sequence typing, L protein mutation identification, cfr plasmid sequence analysis, and *optrA* and *cfr*(B) detection. Isolates M12/0145 and M13/0401 exhibited linezolid MICs of 64 and 16 mg/liter, respectively, and harbored identical 23S rRNA and L22 mutations, but M12/0145 exhibited the mutation in 2/6 23S rRNA alleles, compared to 1/5 in M13/0401. Both isolates were sequence type 22 MRSA staphylococcal cassette chromosome *mec* type IV (ST22-MRSA-IV)/*spa* type t032 isolates, harbored *cfr*, exhibited the PhLOPSA phenotype, and lacked *optrA* and *cfr*(B). They differed by five PFGE bands and 603 SNVs. Isolate M12/0145 harbored *cfr* and *fexA* on a 41-kb conjugative pSCFS3-type plasmid, whereas M13/0401 harbored *cfr* and *lsa(B)* on a novel 27-kb plasmid. This is the first report of *cfr* in the pandemic ST22-MRSA-IV clone. Different *cfr* plasmids and mutations associated with linezolid resistance in genotypically distinct ST22-MRSA-IV isolates highlight that prudent management of linezolid use is essential.

The Emergence and Spread of Multiple Livestock-Associated Clonal Complex 398 Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus Strains among Animals and Humans in the Republic of Ireland, 2010-2014.

Brennan GI, Abbott Y, Burns A, Leonard F, McManus BA, O'Connell B, Coleman DC, Shore AC.

PLoS One. 2016 Feb 17;11(2):e0149396. doi: 10.1371/journal.pone.0149396

Abstract

Clonal complex (CC) 398 methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) are associated with carriage and infection among animals and humans but only a single case of CC398 MRSA has been reported in the Republic of Ireland (ROI). The present study investigated the molecular epidemiology of CC398 MRSA (n = 22) and MSSA (n = 10) from animals and humans in the ROI from 2010-2014. Isolates underwent antimicrobial susceptibility testing, *spa* typing, DNA microarray profiling and PCR for CC398-associated resistance genes. All MRSA underwent SCC*mec* IV or V subtyping. Four distinct CC398-MRSA incidents were identified from (i) a man in a nursing home (spa type t011-SCC*mec* IVa, immune evasion complex (IEC) negative), (ii) a horse and veterinarian who had recently travelled to Belgium (t011-IVa, IEC positive), (iii) pigs (n = 9) and farm workers (n = 9) on two farms, one which had been restocked with German gilts and the other which was a finisher farm (t034-VT, IEC negative, 3/9 pigs; t011-VT, IEC negative, 6/9 pigs & 9/9 farm workers), and (iv) a child who had worked on a pig farm in the UK (t034-VT, IEC negative). Isolates also carried different combinations of multiple resistance genes including *erm*(A), *erm*(B), *tet*(K), *tet*(M) & *tet*(L), *fexA*, *spc*, *dfrG*, *dfrK, aacA-aphD* and *aadD* further highlighting the presence of multiple CC398-MRSA strains. CC398 MSSA were recovered from pigs (n = 8) and humans (n = 2). CC398 MSSA transmission was identified among pigs but zoonotic transmission was not detected with animal and human isolates exhibiting clade-specific traits. This study highlights the importation and zoonotic spread of CC398 MRSA in the ROI and the spread of CC398 MSSA among pigs. Increased surveillance is warranted to prevent further CC398 MRSA importation and spread in a country that was considered CC398 MRSA free.

Evaluation of commercially available chromogenic media for the laboratory detection of methicillin-resistant *Staphylococcus aureus*.

Brennan GI, Herra C, Coleman DC, O'Connell B and Shore AC.

J Hosp Infect (2016) 92; 287-92

Abstract

Background

Selective chromogenic media allowing one-step meticillin-resistant *Staphylococcus aureus* (MRSA) isolation and identification are widely used. However, the changing epidemiology of MRSA means that the suitability of these chromogenic media requires investigation.

Aim

To evaluate the following chromogenic media – Colorex MRSA, MRSA Select II, ChromID MRSA, and MRSA Brilliance 2 – for the detection of divergent strain types.

Methods

We used a diverse collection of *S. aureus*, including strains harbouring the *mecC* gene, strains expressing varying levels of meticillin resistance, and isolates recovered from patient samples.

Findings

MRSA Select II, Colorex MRSA, and ChromID each grew at a density of 1.5×10^1cfu/mL for each SCC*mec* type investigated. Brilliance 2 demonstrated growth at 1.5×10^1cfu/mL for *mecC* MRSA but at a higher density (1.5×10^4cfu/mL) for the three *mecA* MRSA strains. All four media demonstrated excellent sensitivity for MRSA detection (≥99%), but reduced levels of specificity (85–73%) when challenged with a range of meticillin-susceptible *S. aureus* (MSSA) isolates. High levels of false positives (~50%) were also obtained with all chromogenic media when tested with *mec*-negative borderline oxacillin-resistant *S. aureus* (BORSA) isolates.

Conclusion

Although false positives may be obtained with some strains of MSSA and BORSA, the high sensitivity of these media and their ability to recover almost all MRSA tested (including oxacillin-susceptible and *mec*C-positive strains) confirm the value of chromogenic agar in MRSA detection.

Enhanced tracking of the nosocomial transmission of endemic ST22-MRSA-IV among patients and environmental sites using whole-genome sequencing.

Kinnevey PM, Shore AC, Mac Aogáin M, Creamer E, Brennan GI, Humphreys H, Rogers TR, O'Connell B and Coleman DC.

J Clin Microbiol. 2016 Feb;54(2):445-8

Abstract

Whole-genome sequencing (WGS) of 41 patient and environmental sequence type 22 methicillin-resistant *Staphylococcus aureus* staphylococcal cassette chromosome *mec* type IV (ST22-MRSA-IV) isolates recovered over 6 weeks in one acute hospital ward in Dublin, Ireland, where ST22-MRSA IV is endemic, revealed 228 pairwise combinations differing by <40 single nucleotide variants corresponding to potential cross-transmission events (CTEs). In contrast, 15 pairwise combinations of isolates representing five CTEs were previously identified by conventional molecular epidemiological typing. WGS enhanced ST22-MRSA-IV tracking and highlighted potential transmission of MRSA via the hospital environment.

Transmission of methicillin-resistant *Staphylococcus aureus* in long-term care facilities and their related healthcare networks

Harrison EM, Ludden C, Brodrick H, Blane B, Brennan G, Morris D, Coll F, Reuter S, Brown NM, Holmes MA, O'Connell B, Parkhill J, Török ME, Cormican M, Peacock SJ.

Genome Med. 2016 Oct 3;8 (1):102.

Abstract

BACKGROUND: Long-term care facilities (LTCF) are potential reservoirs for methicillin-resistant *Staphylococcus aureus* (MRSA), control of which may reduce MRSA transmission and infection elsewhere in the healthcare system. Whole-genome sequencing (WGS) has been used successfully to understand MRSA epidemiology and transmission in hospitals and has the potential to identify transmission between these and LTCF.

METHODS: Two prospective observational studies of MRSA carriage were conducted in LTCF in England and Ireland. MRSA isolates were whole-genome sequenced and analyzed using established methods. Genomic data were available for MRSA isolated in the local healthcare systems (isolates submitted by hospitals and general practitioners).

RESULTS: We sequenced a total of 181 MRSA isolates from the two study sites. The majority of MRSA were multilocus sequence type (ST)22. WGS identified one likely transmission event between residents in the English LTCF and three putative transmission events in the Irish LTCF. WGS also identified closely related isolates present in colonized Irish residents and their immediate environment. Based on phylogenetic reconstruction, closely related MRSA clades were identified between the LTCF and their healthcare referral network, together with putative MRSA acquisition by LTCF residents during hospital admission.

CONCLUSIONS: These data confirm that MRSA is transmitted between residents of LTCF and is both acquired and transmitted to others in referral hospitals and beyond. Our data present compelling evidence for the importance of environmental contamination in MRSA transmission, reinforcing the importance of environmental cleaning. The use of WGS in this study highlights the need to consider infection control in hospitals and community healthcare facilities as a continuum.

Whole-Genome Sequencing for Routine Pathogen Surveillance in Public Health: a Population Snapshot of Invasive Staphylococcus aureus in Europe.

Aanensen DM, Feil EJ, Holden MT, Dordel J, Yeats CA, Fedosejev A, Goater R Castillo-Ramírez S, Corander J, Colijn C, Chlebowicz MA, Schouls L, Heck M, Pluister G, Ruimy R, Kahlmeter G, Åhman J, Matuschek E, Friedrich AW, Parkhill J, Bentley SD, Spratt BG, Grundmann H; European SRL Working Group.

MBio. 2016 May 5;7(3). pii: e00444-16.

Abstract

The implementation of routine whole-genome sequencing (WGS) promises to transform our ability to monitor the emergence and spread of bacterial pathogens. Here we combined WGS data from 308 invasive *Staphylococcus aureus* isolates corresponding to a pan-European population snapshot, with epidemiological and resistance data. Geospatial visualization of the data is made possible by a generic software tool designed for public health purposes that is available at the project URL (http://www.microreact.org/project/EkUvg9uY?tt=rc). Our analysis demonstrates that high-risk clones can be identified on the basis of population level properties such as clonal relatedness, abundance, and spatial structuring and by inferring virulence and resistance properties on the basis of gene content. We also show that in silico predictions of antibiotic resistance profiles are at least as reliable as phenotypic testing. We argue that this work provides a comprehensive road map illustrating the three vital components for future molecular epidemiological surveillance: (i) large-scale structured surveys, (ii) WGS, and (iii) community-oriented database infrastructure and analysis tools.

IMPORTANCE:

The spread of antibiotic-resistant bacteria is a public health emergency of global concern, threatening medical intervention at every level of health care delivery. Several recent studies have demonstrated the promise of routine whole-genome sequencing (WGS) of bacterial pathogens for epidemiological surveillance, outbreak detection, and infection control. However, as this technology becomes more widely adopted, the key challenges of generating representative national and international data sets and the development of bioinformatic tools to manage and interpret the data become increasingly pertinent. This study provides a road map for the integration of WGS data into routine pathogen surveillance. We emphasize the importance of large-scale routine surveys to provide the population context for more targeted or localized investigation and the development of open-access bioinformatic tools to provide the means to combine and compare independently generated data with publicly available data sets.

POSTERS PRESENTED AT NATIONAL AND INTERNATIONAL CONFERENCES

An in-depth analysis of an emerging CC1-MRSA-IV/t127 clone in Irish hospitals using whole-genome sequencing. Earls, M, Kinnevey PM, Brennan, GI, Slickers, P, O'Connell, B, Humphreys, H, Shore, AC, and Coleman, DC. Presented at the International Symposium on Staphylococci and Staphylococcal Infections, Korea, September 2016.

The prevalence and characterisation of *Staphylococcus aureus* isolates recovered from inmates in an Irish prison. Saab, S, Conroy, E, Brennan, GI and O'Connell, B. Presented at the Pan-Celtic Microbiology Meeting, Dublin, October 2016.

PRESENTATIONS AT NATIONAL CONFERENCES

Panceltic Microbiology Meeting, Dublin, October 2016

- MRSA Outside the Hospital
- Identification of diverse lineages and novel SCC*mec* elements harbouring *fusC* among CA-MRSA isolates in Ireland presented at the Panceltic Microbiology Meeting, Dublin, October 2016.

RESOURCES

Staff

During 2016 the staff working in the NMRSARL were;

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

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

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

Facilities

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

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 € 250,542. As in previous years, shortfall in the laboratory non-pay budget was supplemented from savings achieved through reduction in staffing enabling the laboratory to maintain the level of service for our users.

Administration

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

BIBLIOGRAPHY

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