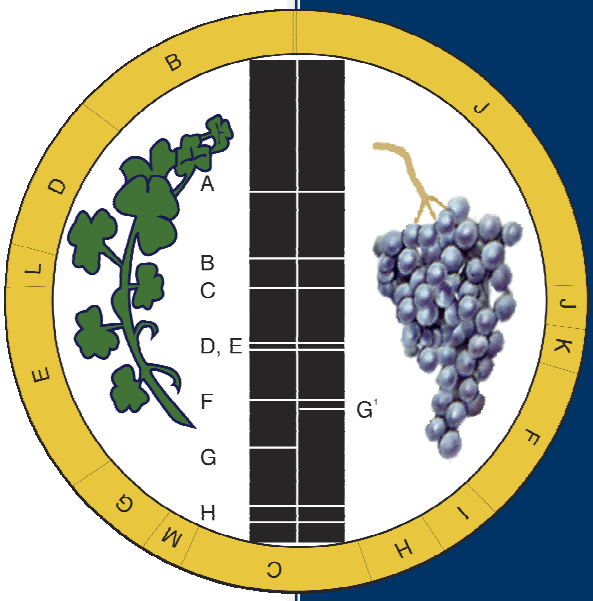
ANNUAL REPORT 2012



National Meticillin-Resistant *Staphylococcus aureus* Reference Laboratory

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Introduction

In 2012 the National meticillin-resistant *Staphylococcus aureus* Reference Laboratory (NMRSARL) continued to provide a high quality service to its users.

The primary role of the NMRSARL is to assist routine microbiology hospitals in the correct identification and control of MRSA using specialized molecular and epidemiological typing techniques.

The annual report for 2012 shows in detail all the achievements and the workload of the laboratory throughout the year along with the involvement of laboratory and clinical staff in education and research aspects of MRSA.

The main achievements of the NMRSARL during 2012 were:

- Involvement in the detection of novel strains of MRSA in Ireland. In particular, NMRSARL contributed to the characterisation of strains of MRSA carrying novel SCC*mec* elements which have recently emerged in Ireland and Europe (1, 2) [1];
- Performing continued surveillance of resistance to glycopeptides (i.e. vancomycin and teicoplanin) and noting continued low levels of resistance which is re-assuring given the importance of these agents in treating serious infection caused by MRSA;
- Monitoring resistance to new antibiotics and noting continued lack of resistance. However, there is a need for continued vigilance to detect emergent resistance;
- Involvement in the recognition of the introduction of new strains of community associated MRSA causing hospital outbreaks and assisted in controlling the outbreak;
- Expansion of service repertoire and the introduction of multiplex real time PCR techniques for the detection of resistance and virulence mechanisms;
- Cost reduction in line with maintaining a quality services;
- The continued strengthening of academic links between the NMRSARL and Trinity College Dublin.

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Clinical Director

Chief Medical Scientist

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
- investigation of resistance
- detection of the *pvl* gene encoding the Panton-Valentine leucocidin toxin
- characterization of selected isolates by staphylococcal protein A (*spa*) typing

Investigation of meticillin susceptible *S. aureus* (MSSA) isolates

- For the detection of *pvl* gene that encodes for the Panton-Valentine leucocidin Toxin
- Outbreak investigation of strains using PFGE

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 NMRSARL..

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.

Administration

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

Routine Laboratory Work

Reference Laboratory Work

In NMRSARL, all requests received are considered incidents and may include requests for information, requests to investigate isolates from potential outbreaks or requests to investigate some other problem with the isolate(s). Figure 1 summarises the number of incidents investigated during 2012 along with the number of tests performed on isolates received within the laboratory.

Briefly, the declining rates of MRSA in Irish hospitals continued in 2012 resulting in a decrease in the number of isolates investigated under the EARS-Net project. In contrast however, the reference laboratory workload increased particularly with regards to more specialised techniques including *spa* typing and molecular detection tests.

The introduction of a triplex assay for the detection of *mecA,* PVL and *nuc* meant that there was an increase in the number of *mecA* and *nuc* investigations completed. The amount of information obtained from *spa* typing of isolates is invaluable in assisting in the monitoring of strains throughout Ireland and the early detection of emerging strains.

European Antimicrobial Resistance Surveillance Network (EARS-Net)

Twenty-nine laboratories throughout Ireland submitted 200 MRSA isolates causing blood-stream infections to NMRSARL during 2012 for monitoring of epidemiological types and resistance to clinically significant antimicrobials. This laboratory work is performed for the EARS-Net project, a European initiative that, in Ireland, is managed through the Health Protection Surveillance Centre (HPSC).

All isolates undergo Antibiogram-Resistogram (AR) typing using the Clinical and Laboratory Standards Institute (CLSI) methodology using a panel of 23 antimicrobial agents. From this data the NMRSARL provides HPSC with information on rates of resistance to clinically useful antibiotics. The oxacillin minimum inhibitory concentration (MIC) is determined and isolates are screened for reduced susceptibility to vancomycin and teicoplanin using the E-test™ macro-method along with screening agar plates containing vancomycin and teicoplanin.

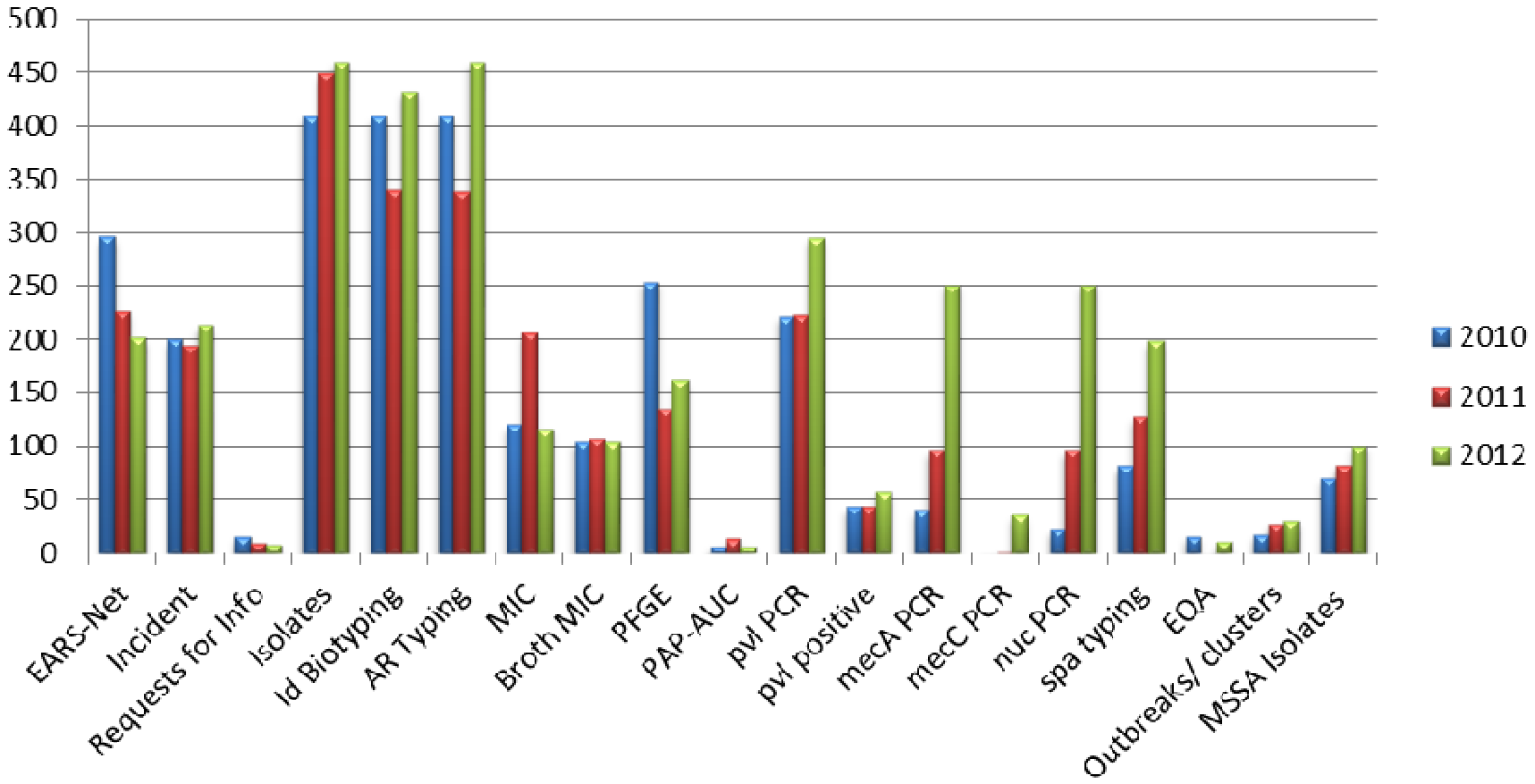

Figure 1: Routine workload of the National MRSA Reference Laboratory in 2012 compared with that of the previous years. *pvl*, Panton-Valentine leukocidin toxin gene; *mecA*, gene encoding meticillin resistance; *mecC,* gene encoding meticillin resistance *spa*, staphylococcal protein A gene typing; PFGE, pulsed field gel electrophoresis. Id Biotyping, MIC, AR Typing and PFGE is performed all EARS-Net isolates.

Monitoring resistance among Irish strains of MRSA

Epidemiological typing of MRSA isolates investigated under the EARS-Net project using AR typing enables the NMRSARL to monitor resistance among MRSA strains against clinically useful antimicrobial agents and to identify emerging resistance that may cause concern into the future. 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 and enhanced surveillance of these strains is essential in order to ensure that these strains do not spread into Irish hospitals.

Fusidic Acid Resistance

Monitoring antimicrobial resistance in MRSA isolates investigated under the EARS-Net project has shown that resistance to fusidic acid increased from <10% between 1999 and 2001 to 27% in 2006. This increase continued through to 2010 and, while a 7% reduction was seen in 2011, the increasing trend returned in 2012 with 40.5% (81/200) of all EARS-Net isolates exhibiting resistance.

This increasing trend over 12 years is a worrying development as fusidic acid remains a clinically useful antimicrobial for difficult to treat skin and soft tissue infections. NMRSARL is collaborating with colleagues in the Dublin Dental University Hospital (DDUH) to further investigate the mechanism of resistance in these isolates.

Glycopeptide Resistance

Vancomycin and teicoplanin remain the mainstay for the treatment of serious infection caused by MRSA. There has been increasing concern about the development of resistance to these agents over recent years and laboratory detection of resistance is complex.

NMRSARL screens all isolates for reduced susceptibility to glycopeptides using agar screening methods. EARS-Net MRSA isolates are also investigated by a commercial MIC system using a macro-method which screens for hetero-glycopeptide resistant *S. aureus* (hGISA). Isolates yielding positive results in the E-test macro-method are confirmed as hGISA by population analysis profile-area under the curve (PAP-AUC) ratio determination.

During 2012, no EARS-Net isolates exhibited reduced susceptibility to glycopeptides. Six reference laboratory isolates were further investigated by PAP-AUC and one was confirmed as hGISA.

Mupirocin Resistance

Mupirocin is the agent of choice to eradicate nasal colonisation with MRSA. The detection of resistance among MRSA strains circulating in Irish is of importance as increasing prevalence will undoubtedly have a major impact on the success of decolonisation, a major strategy in preventing the spread of MRSA.

High-level mupirocin resistance (Hi-MupR) is a common feature among older strains of MRSA in Irish hospitals. Since 2008 NMRSARL has been monitoring the emergence of resistance among MRSA isolates exhibiting an unfamiliar AR pattern which includes resistance to the aminoglycosides gentamicin, kanamycin and tobramycin but with PFG-01 patterns which are associated with the AR06 AR type.

During 2012, this Hi-MupR strain was not detected however low level resistance was detected in 3.6% (8/220) of MRSA isolates from blood; five of these isolates were recognised as an 'old' MRSA strains while the remaining isolates represent isolates exhibiting variant AR06 patterns with low level resistance to mupirocin and with PFG-01 patterns associated with the AR06 AR type.

Collaborative work involving NMRSARL and the DDUH includes investigating the Hi-MupR – conferring plasmids in these isolates.

Linezolid, Quinupristin/ Dalfopristin, Daptomycin and Tigecycline Susceptibility Testing

Monitoring of these newer agents for treatment of MRSA infection is important, as resistance detection is rarely carried out and difficult with not all diagnostic laboratories having the capability to perform appropriate tests.

During 2012, 100 MRSA isolates (from the EARS-Net study) were tested for susceptibility to quinopristin/dalfopristin, daptomycin and tigecycline by E-test MIC determination along with quinopristin/dalfopristin and tigecycline by disk diffusion. All isolates were susceptible.

In addition to this, all isolates submitted to the laboratory were tested against linezolid. Two isolates recovered from the same patient exhibited resistance to linezolid and carried *cfr*, the gene encoding linezolid resistance.

Resistance to linezolid among MRSA strains is unusual and we are working with our research colleagues in determining if the plasmid carrying *cfr* is similar to those previously reported (3).

Molecular Epidemiological Typing

Pulsed Field Gel Electrophoresis (PFGE)

In NMRSARL, the banding pattern obtained by PFGE is assigned a five-digit PFGE type (PFT) number and apparently-related groups of PFTs are grouped together according to the criteria of Tenover *et. al. (4, 5).*

AR typing and PFGE typing results are combined to yield AR-PFG types.

Prior to 2012, the NMRSARL typed all isolates submitted under the EARS-Net project by PFGE. Although inexpensive, PFGE is laborious and the comparison of results between different laboratories is only possible if both laboratories use the same techniques. In addition, while PFGE provides information about the genetic relatedness of isolates, limited information about the epidemiological type of isolates, in particular with regards to sporadically occurring MRSA strains, can be deduced from some banding patterns.

PFGE patterns of AR-PFG 06-01 isolates have tended to be relatively homogeneous. When Irish laboratories first participated in EARS-Net in 1999, just seven PFGE patterns were detected among the AR-PFG 06-01 isolates investigated. However with the development of the PFGE method used the number of patterns recognised has significantly increased to over 180 PFTs.

Since 2001 three patterns have predominated and this trend continued in 2011, with 33.2% (76/225) of isolates exhibiting one of three closely-related patterns, PFT 01018, 01039 and 01042. For this reason in 2012 the NMRSARL reduced the number of EARS-Net isolates investigated by PFGE and limited it mainly to investigate outbreak situations in hospital settings. A proportion of EARS-Net isolates continue to be investigated to ensure that there are no chromosomal changes occurring the MRSA population that would be missed using only AR typing.

Among EARS-Net isolates investigated by PFGE in 2012 the AR-PFG 06-01 pattern continued to predominate.

spa Typing

DNA sequencing of the Staphylococcal protein A (*spa*) gene is a well-established discriminatory method for outbreak investigations. It has also been shown that this region reflects long-term epidemiology.

spa typing recognises mutations or repeat insertion/deletion events that can cause changes in the polymorphic X region of the *spa* gene. The availability of MLST data associated with *spa* types on an online database facilitates comparison of Irish isolates with isolates from all other countries.

Of all isolates submitted to the NMRSARL during 2012, 206 were investigated by *spa* typing . This analysis included a selection of EARS-net isolates and all PVL-positive isolates and some PVL- negative isolates that exhibited an AR pattern similar to that of a non-multi-antibiotic resistant pattern, described as AR06. These isolates are not assigned to an AR type as they are urease positive but instead are termed as 'No Type' ('NT') because experience in NMRSARL with such isolates has shown that these isolates cannot be reliably typed by AR typing and required additional investigation (6).

Based Upon Repeating Patterns (BURP) analysis clusters *spa* types based on the repeat succession pattern of *spa* types and has shown to have good concordance with MLST data. BURP analysis of all isolates investigated during 2012 is shown in Figure 1.

There were 24 different *spa* types recognised among 54 PVL-positive isolates, fourteen of which was for only one isolate. Among the remaining isolates there were some predominant *spa* types with *spa* type t852 (ST22) accounting for 13% (7/54) of the isolates while t008 (ST8) accounted for 11% (6/54).

spa type t852 is associated with ST22 and, while the PVL-negative ST22-MRSA-IV is the pandemic strain in Irish hospitals, this PVL positive strain is widely associated with India and, along with ST772-MRSA-V, has displaced previously predominant nosocomial strains in Indian hospitals and is closely related to other *spa* types recognised during 2012- t005 (2/54) and t5422 (1/54).

spa type t008 (also known as USA 300) has been recognised among Irish PVL positive isolates since 2007 with the highest number of isolates of this spa type recovered in 2009 (29%, 11/38 isolates). While this strain has been associated with a number of nosocomial outbreaks in the United States, to the best of our knowledge, no such incidents have occurred in Ireland.

Other sequence types recognised among the PVL positive strains included ST1, ST30, S88, ST93 and as in 2011, *spa* type t657 (the Bengal Bay clone) was also recognised among a number of isolates (n=5).

The PVL-negative isolates (n=143) exhibited much greater diversity. Among these isolates investigated there were 58 different *spa* types with 36 of these recognised in only one isolate.

The most frequently occurring *spa* type among PVL negative isolates was t002, associated with ST5 (15%, 22/143).

spa type t878 associated with ST779, which has been reported to carry a novel pseudo SCC*mec*-SCC-SCCCRISPR element continued to be detected in Irish hospitals and was recognised in three isolates.

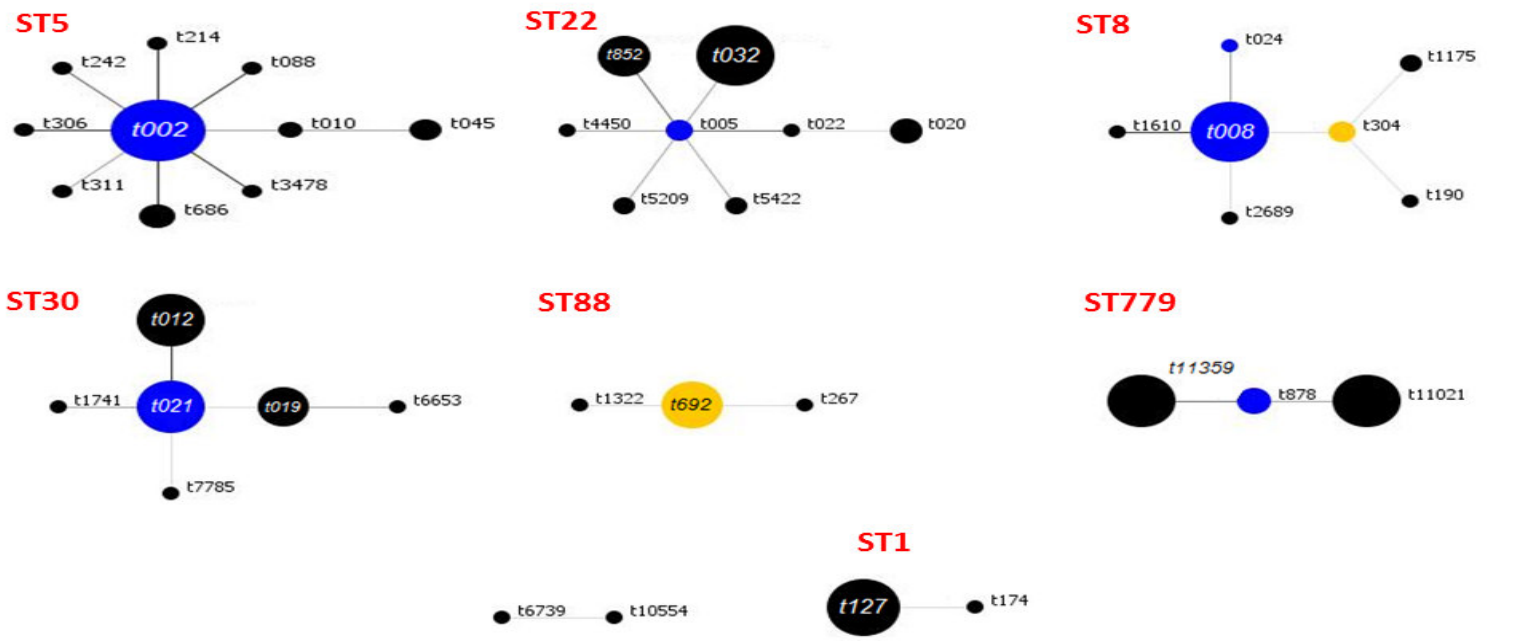

Figure 2: Population snapshot based on BURP analysis of all *S. aureus* isolates that were *spa* typed during 2012. BURP parameters excluded *spa* types with less than five repeats and clustered *spa* types into *spa* clonal complexes (*spa*-CC) if the cost distance was less than four. Analysis resulted in 8 *spa* CCs, 16 singletons, and 9 excluded *spa* types. *spa* types with less than five repeats. Each dot represents a unique *spa* type. The diameter of a dot is proportional to the number of isolates of the corresponding *spa* type. Blue dots represent group founders, defined as the *spa* type(s) with the highest founder score within a CC while yellow dots show a co-founder. Note that the spacing between linked *spa* types and between unlinked *spa* types and *spa* CCs provides no information concerning the genetic distance between them. *spa* inferred MLST types are shown in red.

Monitoring the Epidemic Strains Prevalent in the Irish MRSA Population

Through the epidemiological data obtained from isolates submitted to the EARS-Net project, NMRSARL monitors MRSA strains that are circulating in Irish hospitals.

Previously we have shown that the majority of MRSA isolates recovered in Irish hospitals between 1971 and 2003 belonged to one of seven internationally spread MRSA clones (ST239, ST247, ST250, ST5, ST22, ST36 and ST8) and had SCC*mec* types I, Ia, II, III or IV but ST8 isolates showed an unexpected degree of diversity within the SCC*mec* element. Since 2004, NMRSARL has combined AR typing results with PFGE typing results to generate AR-PFG types (4). Figure 3 shows how the AR-PFG type distribution in participating hospitals has changed since 1999 and also includes data on multilocus sequence typing (MLST) and staphylococcal cassette chromosome (SCC) *mec* typing extrapolated from a study completed in 2004 (6).

Among MRSA isolates recovered from blood, the prevalence of one AR-PFG type 06-01 increased from 22% in 1999 to 81.2%% in 2010 but reduced to 74.6% in 2011. In 2012 this strain was once again associated with 85% of isolates submitted for investigation to the NMRSARL.

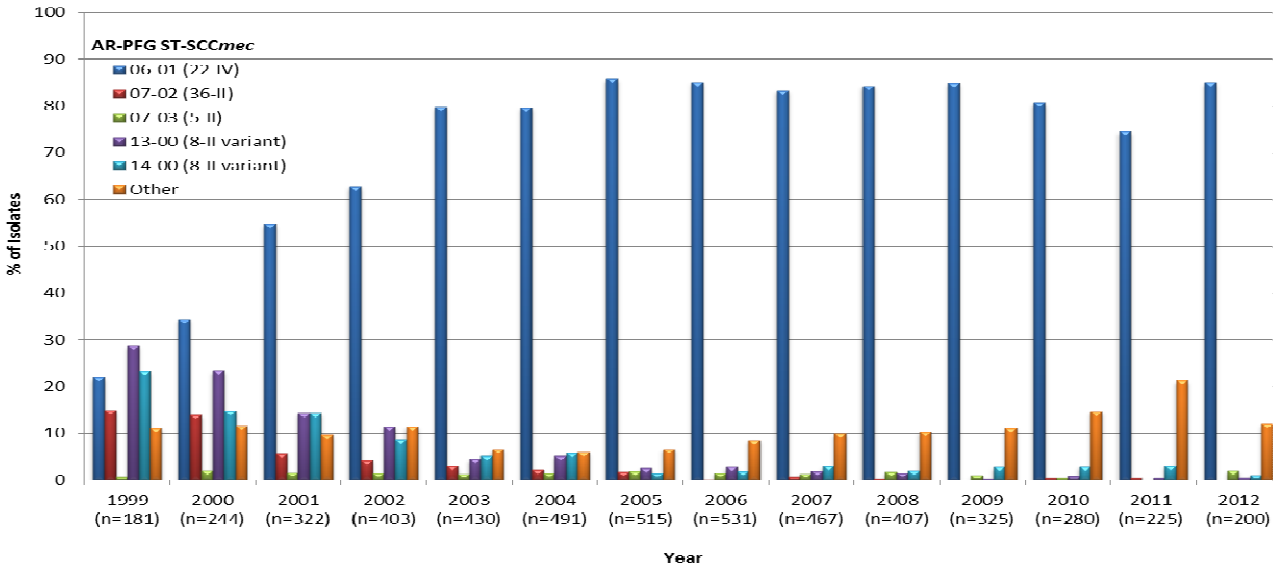

Figure **3: Epidemiological type distribution (%) among MRSA isolates in Irish hospitals that participate in the European Antimicrobial Resistance Surveillance Network (1999 to 2011).**

AR-PFG, antibiogram-resistogram type and pulsed field gel electrophoresis group; ST-SCC*mec*, MLST sequence type and SCC*mec* type (SCC*mec* II variant, variants of SCC*mec* type II).

Panton-Valentine Leukocidin (PVL) Carriage in MRSA in Ireland

In a study investigating PVL in MRSA in Ireland, carriage of PVL was shown to be unreliable as a sole marker for community-acquired MRSA (CA-MRSA).

Molecular characterization of PVL-positive MRSA isolates confirmed that all isolates carried SCC*mec* type IV but six genotypes (ST30, ST8, ST22, ST80, ST5 and ST154) were represented among these isolates. Epidemiological data showed that 36% of patients in Ireland from whom PVL-positive isolates were recovered were of non-Irish ethnic origin (7).

In 2012, 301 MRSA and meticillin-susceptible *S. aureus* (MSSA) isolates were tested for carriage of PVL on the basis of clinical or epidemiological need. These comprised 29 EARS-Net MRSA isolates, 83 MSSA and 189 MRSA isolates. Thirteen percent of MSSA (11/83) and 23% of MRSA (50/218) were PVL-positive. Additional molecular epidemiology information is available in the *spa* typing section above.

MRSA Associated with Animals

During 2012 the NMRSARL reported on the first case of the most well-known livestock associated MRSA strain- ST398 recovered in an Irish patient. This strain was originally reported in the Netherlands where it was recovered from pigs and pig farmers and has since been associated with cattle, sheep and chickens. This strain has been recognised in several European countries as well as in the USA and Canada.

The report of the discovery of *mecC*, the gene encoding low-level resistance is of great concern and, while originally reported in strains from Ireland, England, Scotland and Denmark it has now been widely reported throughout Europe and is mainly associated with animals

The NMRSARL have alerted hospital microbiology staff of the difficulties in detecting this strain of MRSA due to the low level oxacillin resistance it exhibits. The availability of molecular tests in the NMRSARL for the detection of *mecC* continues to assist any users who have encountered difficulties in distinguishing between *mecA* and *mecC* encoding MRSA and MSSA.

Quality Management System

NMRSARL is fully accredited under Clinical Pathology Accreditation (CPA) standards incorporating ISO 15189. The quality management system in place within NMRSARL ensures that there are co-ordinated activities in place on order to continually improve the effectiveness and efficiency of the laboratory. These include internal and external audits, document reviews, key performance indicators (quality indicators) and regular communication with users of the laboratory.

During 2012 the laboratory successfully completed an inspection by CPA assessors where no non-conformances were identified against the standards. This CPA assessment was the last by CPA assessors and the laboratory has applied for accreditation with the Irish National Accreditation Board in accordance with ISO15189 standards for medical laboratories.

Quality Objectives

During 2012 the NMRSARL set quality objectives relating to user requirements, staff training and education, health and safety and waste management.

Progress report on user related Quality Objectives

1. Introduction of real-time PCR assay for the detection of *eta, etb* and *etd* in *S. aureus* isolates.

A student completing an MSc in Clinical Laboratory Science has undertaken this research project and the laboratory expects to introduce this service to users in late 2013.

2. Epidemiological typing of MSSA isolates by PFGE and where necessary *spa* typing.

Staff completed a validation of the investigation of MSSA isolates by PFGE and this service can now be offered to users who are investigating a cluster of MSSA isolates.

spa typing is also available if the PFGE results provides insufficient data to assist the user in their outbreak investigation and an increase in *spa* typing of MSSA isolates has also led to a greater understanding of the strains circulating in Ireland.

Resources

Staff

The staffing complement of the NMRSARL consisted of a Chief Medical Scientist (Ms. Gráinne Brennan), a Molecular Microbiologist Dr. Pamela Morgan), a Basic Grade Medical Scientist (Ms. Emma Gibbons) and a Medical Laboratory Aide (Mr. Paul Grier). During 2012 the Molecular Microbiologist and the Basic Grade Medical Scientist were on extended leave of absences and in their absence a Medical Scientist (Ms. Tanya Fleming) was seconded from the routine microbiology department while Mr. James Kerins was also recruited as a Medical Scientist.

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 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 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 amounts to €260,348 representing a 5% reduction on the previous year. While some of the offset was managed through reduction in staffing, it presented a difficult challenge in maintaining the level of service for our users.

Education

Internal Training at NMRSARL

NMRSARL plays a prominent role in the education of laboratory staff, doctors and nurses throughout Ireland and this is achieved by regular feedback, presentations and reports.

In particular, NMRSARL staff gave lectures to undergraduate and postgraduate microbiology students in the Moyne Institute, TCD, the Department of Clinical Microbiology, TCD, and Dublin Institute of Technology and also made oral presentations at SJH Grand Rounds. Scientific staff shared techniques used in NMRSARL with staff from other hospital laboratories, research facilities and transition year students and provided expert knowledge to students of other laboratories completing higher degrees.

NMRSARL staff completed internal training courses in areas such as fire safety, chemical safety, manual handling, waste management, transport of patient specimens, computer skills, management skills, the quality management system and attended journal clubs organized within St. James's Hospital. One member of staff continued through second year of the MSc in Clinical Laboratory Science through DIT, Kevin Street. Another member of staff started a research PhD on the characterisation of sporadically occurring MRSA in Ireland through the Dublin Dental Hospital, TCD. The laboratory also facilitated a post graduate student from Trinity College in completion of a research project.

Meetings attended by NMRSARL Staff

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:

- International Symposium on Staphylococci and Staphylococcal infections, Lyon, France
- Focus on Infection, TCD
- Dublin Academy of PathogenomIcs and Infections Biology (DAPI), UCD, Dublin
- BioMedica, RDS, Dublin
- Academy of Medical Laboratory Science, Microbiology Advisory Body Meetings

Research

Internal Research Projects

1. The monitoring of reduced susceptibility to glycopeptide among Irish MRSA isolates
2. Replacing current North American Clinical and Laboratory Standards Institute (CLSI) susceptibility techniques in NMRSARL with European Committee on Antimicrobial Susceptibility Testing (EUCAST) techniques and determining the implication this change will have on AR patterns.
3. The detection of the recently described new SCC*mec* element (SCC*mec*XI) harboring *mecC* in MRSA isolates from Irish hospitals
4. The development of a real time PCR assay for the detection of exfoliative toxins in *S. aureus* isolates.
5. Epidemiological typing of MSSA isolates causing BSI in Irish patients
6. Investigation of coagulase negative Staphylococci and MRSA recovered from nasal swabs.

Collaboration with Other Irish Reference Laboratories

NMRSARL maintains contact with colleagues in other Irish Reference Laboratories such as the Epidemiology and Molecular Biology Unit and the Meningococcal Reference Laboratory.

Committees

NMRSARL staff members sit on the Irish EARS-Net Steering Committee, the European Staphylococcal Reference Laboratories Working Group and on the Royal College of Physicians of Ireland's Policy Group on HealthCare-Associated Infection.

National Collaborative Work

The work described below has been undertaken in collaboration with Professor David Coleman, Dr. Anna Shore and their team at the Dublin Dental School, TCD:

1. Following a study that investigated the genotypes and SCC*mec* types and more recently the virulence and resistance genes of the predominant strains of MRSA in Ireland between 1971 and 2004, research is now focusing on the genotypes, SCC*mec* elements, virulence and resistance genes in infrequently-occurring, 'sporadic' and/or unusual MRSA isolates.

2. Investigation of the genetic mechanism of fusidic acid resistance in MRSA in Ireland.

3. Investigation of the usefulness of a *S. aureus* DNA microarray for genotyping MRSA isolates in Ireland and for enhancing discrimination and tracking of MRSA.

4. Characterisation of the genotypes, virulence and antimicrobial resistance genes of *pv*l-positive MRSA in Ireland.

5. Investigation of MRSA from animal populations for the presence of *mecC* in order to determine if isolates harboring this gene are a significant problem among MRSA isolates from animals in Ireland, or if the zoonotic spread of MRSA with this *mecC* are contributing to the burden of MRSA among humans.

6. Monitoring of the characteristics of novel and potentially emerging MRSA clones e.g. ST772-MRSA-V, and the evolution of existing MRSA clones in Irish hospitals and communities, such as subpopulations of ST22-MRSA-IV with enhanced virulence or extended antimicrobial resistance potential.

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

International Collaboration

In 2006, NMRSARL participated in the EARS-Net *spa* typing project and the results of the work were published in January 2010.

The second phase of this project began in January 2011 with the collection of isolates. The aim of this is project to monitor any changes that may have occurred among the prevalent strains. The laboratory, with the support of the Dublin Dental Hospital, submitted data on the *spa* typing of these isolates, to the National Institute for Public Health and Environment, The Netherlands, who were co-ordinating the data from all participating European countries.

Discussions with staff from the Scottish, Danish and UK Reference Laboratories to organize an external quality assessment (EQA) programme between the four centers continued throughout 2012 and successfully resulted in the development of a suitable isolate exchange programme between the laboratories. The three laboratories also continue to provide assistance to each other on topics relating to MRSA specific to Ireland, England and Scotland such as local resistance and epidemiology of MRSA.

Publications involving staff of the NMRSARL

Emergence of hospital- and community-associated panton-valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit

J Clin Microbiol. 2012 Mar; 50 (3):841-7.

Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O'Connell B.

Abstract

Sequence type 22 (ST22) methicillin-resistant *Staphylococcus aureus* (MRSA) harboring staphylococcal cassette chromosome *mec* (SCC*mec*) IV (ST22-MRSA-IV) has predominated in Irish hospitals since the late 1990s. Six distinct clones of community-associated MRSA (CA-MRSA) have also been identified in Ireland. A new strain of CA-MRSA, ST772-MRSA-V, has recently emerged and become widespread in India and has spread into hospitals. In the present study, highly similar MRSA isolates were recovered from seven colonized neonates in a neonatal intensive care unit (NICU) in a maternity hospital in Ireland during 2010 and 2011, two colonized NICU staff, one of their colonized children, and a NICU environmental site. The isolates exhibited multi-antibiotic resistance, spa type t657, and were assigned to ST772-MRSA-V by DNA microarray profiling. All isolates encoded resistance to macrolides [*msr(A)* and *mpb(BM)*] and aminoglycosides (*aacA-aphD* and *aphA3*) and harbored the Panton-Valentine leukocidin toxin genes (*lukF-PV* and *lukS-PV*), enterotoxin genes (*sea, sec, sel,* and egc), and one of the immune evasion complex genes (*scn*). One of the NICU staff colonized by ST772-MRSA-V was identified as the probable index case, based on recent travel to India. Seven additional hospital and CA-ST772-MRSA-V isolates recovered from skin and soft tissue infections in Ireland between 2009 and 2011 exhibiting highly similar phenotypic and genotypic characteristics to the NICU isolates were also identified. The clinical details of four of these patients revealed connections with India through ethnic background or travel. Our study indicates that hospital-acquired and CA-ST772-MRSA-V is currently emerging in Ireland and may have been imported from India on several occasions.

DNA microarray profiling of a diverse collection of nosocomial methicillin-resistant *Staphylococcus aureus* isolates assigns the majority to the correct sequence type and staphylococcal cassette chromosome *mec* (SCC*mec*) type and results in the subsequent identification and characterization of novel SCC*mec*-SCCM1 composite islands.

Antimicrob Agents Chemother. 2012 Oct; 56 (10):5340-55.

Shore AC, Brennan OM, Deasy EC, Rossney AS, Kinnevey PM, Ehricht R, Monecke S, Coleman DC.

Abstract

One hundred seventy-five isolates representative of methicillin-resistant *Staphylococcus aureus* (MRSA) clones that predominated in Irish hospitals between 1971 and 2004 and that previously underwent multilocus sequence typing (MLST) and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were characterized by *spa* typing (175 isolates) and DNA microarray profiling (107 isolates). The isolates belonged to 26 sequence type (ST)-SCC*mec* types and subtypes and 35 *spa* types. The array assigned all isolates to the correct MLST clonal complex (CC), and 94% (100/107) were assigned an ST, with 98% (98/100) correlating with MLST. The array assigned all isolates to the correct SCC*mec* type, but subtyping of only some SCC*mec* elements was possible. Additional SCC*mec*/SCC genes or DNA sequence variation not detected by SCC*mec* typing was detected by array profiling, including the SCC-fusidic acid resistance determinant *Q6GD50/fusC*. Novel SCC*mec*/SCC composite islands (CIs) were detected among CC8 isolates and comprised SCC*mec* IIA-IIE, IVE, IVF, or IVg and a ccrAB4-SCC element with 99% DNA sequence identity to SCC(M1) from ST8/t024-MRSA, SCCmec VIII, and SCC-CI in *Staphylococcus epidermidis.* The array showed that the majority of isolates harbored one or more superantigen (94%; 100/107) and immune evasion cluster (91%; 97/107) genes. Apart from fusidic acid and trimethoprim resistance, the correlation between isolate antimicrobial resistance phenotype and the presence of specific resistance genes was ≥97%. Array profiling allowed high-throughput, accurate assignment of MRSA to CCs/STs and SCC*mec* types and provided further evidence of the diversity of SCC*mec*/SCC. In most cases, array profiling can accurately predict the resistance phenotype of an isolate.

First Irish report of livestock-associated MRSA strain

Gráinne I. Brennan, Brian O' Connell, David C. Coleman, Anna C. Shore

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Transmission of endemic ST22-MRSA-IV on four acute hospital wards investigated using a combination of *spa*, *dru* and pulsed-field gel electrophoresis typing

Creamer E, Shore AC, Rossney AS, Dolan A, Sherlock O, Fitzgerald-Hughes D, Sullivan DJ, Kinnevey PM, O'Lorcain P, Cunney R, Coleman DC, Humphreys H.

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Abstract

The transmission of meticillin-resistant *Staphylococcus aureus* (MRSA) between individual patients is difficult to track in institutions where MRSA is endemic. We investigated the transmission of MRSA where ST22-MRSA-IV is endemic on four wards using demographic data, patient and environmental screening, and molecular typing of isolates. A total of 939 patients were screened, 636 within 72 h of admission (on admission) and 303 >72 h after admission, and 1,252 environmental samples were obtained. Isolates were typed by spa, dru and pulsed-field gel electrophoresis (PFGE) typing. A composite dendrogram generated from the three sets of typing data was used to divide isolates into 'dendrogram groups' (DGs). Ten percent of patients (92/939) were MRSA-positive; 7 % (44/636) on admission and 16 % (48/303) >72 h after admission (p = 0.0007). MRSA was recovered from 5 % of environmental specimens (65/1,252). Most isolates from patients (97 %, 85/88) and the environment (97 %, 63/65) exhibited the ST22-MRSA-IV genotype. Four DGs (DG1, DG4, DG16 and DG17) accounted for 58 % of ST22-MRSA-IV isolates from patients. Epidemiological evidence suggested cross-transmission among 44/92 patients (48 %) but molecular typing confirmed probable cross-transmission in only 11 instances (13 %, 11/88), with the majority of cross-transmission (64 %; 7/11) occurring on one ward. In the setting of highly clonal endemic MRSA, the combination of local epidemiology, PFGE, *spa* and *dru* typing provided valuable insights into MRSA transmission.

Emergence of sequence type 779 methicillin-resistant *Staphylococcus aureus* harboring a novel pseudo staphylococcal cassette chromosome *mec* (SCC*mec*)-SCC-SCCCRISPR composite element in Irish hospitals

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Kinnevey PM, Shore AC, Brennan GI, Sullivan DJ, Ehricht R, Monecke S, Slickers P, Coleman DC.

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a major cause of nosocomial infection in Irish hospitals for 4 decades, and replacement of predominant MRSA clones has occurred several times. An MRSA isolate recovered in 2006 as part of a larger study of sporadic MRSA exhibited a rare *spa* (t878) and multilocus sequence (ST779) type and was nontypeable by PCR- and DNA microarray-based staphylococcal cassette chromosome *mec* (SCC*mec*) element typing. Whole-genome sequencing revealed the presence of a novel 51-kb composite island (CI) element with three distinct domains, each flanked by direct repeat and inverted repeat sequences, including (i) a pseudo SCC*mec* element (16.3 kb) carrying *mecA* with a novel *mec* class region, a fusidic acid resistance gene (*fusC*), and two copper resistance genes (*copB* and *copC*) but lacking *ccr* genes; (ii) an SCC element (17.5 kb) carrying a novel ccrAB4 allele; and (iii) an SCC element (17.4 kb) carrying a novel ccrC allele and a clustered regularly interspaced short palindromic repeat (CRISPR) region. The novel CI was subsequently identified by PCR in an additional 13 t878/ST779 MRSA isolates, six from bloodstream infections, recovered between 2006 and 2011 in 11 hospitals. Analysis of open reading frames (ORFs) carried by the CI showed amino acid sequence similarity of 44 to 100% to ORFs from *S. aureus* and coagulase-negative staphylococci (CoNS). These findings provide further evidence of genetic transfer between S. aureus and CoNS and show how this contributes to the emergence of novel SCCmec elements and MRSA strains. Ongoing surveillance of this MRSA strain is warranted and will require updating of currently used SCC*mec* typing methods.

Posters involving staff of the NMRSARL

Ongoing Surveillance of the Emergence and Evolution of *pvl*-Positive ST772-MRSA-V in Ireland

Gráinne I. Brennan, Sarah Tecklenborg, Brian O'Connell, David C. Coleman and Anna C. Shore

P13-182 Presented at the International Symposium on Staphylococci and Staphylococcal Infections, 26th-30th August 2012, Lyon, France

Introduction. Panton-Valentine leukocidin-positive ST772-MRSA-V has recently emerged and become widespread in India. In Ireland, between August 2009 and June 2011 this clone was associated with a cluster in a neonatal intensive care unit (NICU) and several other sporadic nosocomial and community cases.

Objective. To characterise ST772-MRSA-V isolates recovered in Ireland since November 2011 and to compare these with previously recovered ST772-MRSA-V in Ireland.

Methods. Nine isolates submitted to the Irish National MRSA Reference Laboratory since November 2011 were selected for further investigation based on a similar antibiogram-resistogram pattern to previously reported ST772-MRSA-V isolates. Seven isolates were recovered during an outbreak in a NICU in a different hospital to the previously reported ST772-MRSA-V cluster. Two isolates were recovered from patients in the community, one from an Indian male with a history of skin abscesses and the other from a leg abscess of an Irish male with no known Indian connection but with a history of intravenous drug abuse. The isolates underwent *spa* typing and DNA microarray analysis.

Results. All isolates exhibited *spa* type t657 and DNA microarray analysis assigned isolates to CC1 and ST573/772. Isolates were positive for *mecA* but no *ccr* genes were detected. Similar to previously reported ST772 MRSA from Ireland these isolates harboured (1) genes encoding resistance to macrolides and aminoglycosides, (2) the PVL genes *lukF-PV* and *lukS-PV*, (3) the enterotoxin genes *sea*, *sec* and *sel* and the enterotoxin gene cluster, (4) the immune evasion complex gene *scn* but the truncated beta-hemolysin gene was not detected.

Conclusion. The number of ST772-MRSA-V isolates recovered in Ireland and the involvement of this strain in outbreaks in two different NICU is a worry. Molecular epidemiological analysis of ST772-MRSA-V isolates recovered in Ireland suggests multiple importations of this strain. Enhanced surveillance to identify and control the spread of this clone in Irish hospitals is essential.

The changing molecular epidemiology of PVL-positive *Staphylococcus aureus* in Ireland

Sarah Tecklenborg, Anna Shore, Grainne Brennan, Stefan Monecke, Ralf Ehricht, Brian O'Connell, David Coleman

P13-193 Presented at the International Symposium on Staphylococci and Staphylococcal Infections, 26th-30th August 2012, Lyon, France

Aims: Expression of the Panton-Valentine leukocidin (PVL) cytolytic toxin has been linked with community-associated methicillin-resistant *Staphylococcus aureus* (MRSA). A previous study of *pvl*-positive MRSA in Ireland between1999 and 2005 identified 25 isolates belonging to six genotypes. The aim of the present study was to undertake a molecular analysis of *pvl*-positive *S. aureus* isolates recovered in Ireland between 2006 and 2011.

Methodology: 183 *pvl*-positive *S. aureus* isolates, including 152 MRSA and 31 methicillin-susceptible *S. aureus* (MSSA), identified by the Irish National MRSA Reference Laboratory between 2006 and 2011 were investigated by DNA microarray genotyping (Alere, Germany). Clinical details were available for 123/183 isolates.

Results & conclusions: The *pvl*-positive MRSA and MSSA were assigned to 13 and five genotypes, respectively. The dominant clones among MRSA included ST8-IV (32%), ST30-IV (19%), CC80-IV (16%), ST772-V (14%) and ST22-IV (6%). CC30 (35%) predominated among MSSA, followed by CC22 (23%), CC121 (19%), CC1 (19%), and CC78 (10%). Different MRSA clones prevailed at different times including ST8-IV and ST30-IV from 2006-2009 and ST772-MRSA-V from 2009-2011.The prevalence of CC80-IV declined from 47% in 2007 to 0% in 2011. The most common infection associated with the *pvl*-positive *S. aureus* was skin and soft tissue infections (44%), but more serious infections such as sepsis (12%), necrotising pneumonia (2%) and fasciitis (1%) were also identified.

The *pvl*-positive isolates harboured a range of antimicrobial resistance and virulence genes including *blaZ*(91%)*, fosB* (65%), *msr(A)* (32%), *mpbBM* (32%), *aacA-aphD* (20%), *tet*(K) (20%), *erm(C)* (10%), *dfrS1* (7%), *qacC* (2%), *mupA* (1%), immune evasion complex genes (98%), the arginine catabolic mobile element (27%) and toxin genes *egc* (45%), *sea* (17%), *etD* (13%), *sec & sel* (11%) and *tst* (4%). MSSA isolates harboured significantly less virulence and antimicrobial resistance genes than MRSA.

This study identified diverse *pvl*-positive *S. aureus* strains in Ireland with changes in the dominant *pvl*-positive MRSA clones over time, possibly due to introduction of strains by travel or immigration.

Emergence of a Distinct Methicillin-Resistant *Staphylococcus aureus* (MRSA) Lineage Harbouring a Novel Pseudo SCC*mec*-SCC Composite Element in Ireland

Peter Kinnevey, Anna Shore, Gráinne Brennan, Peter Slickers, Stefan Monecke, Ralf Ehricht, David C. Coleman

P13-182 Presented at the International Symposium on Staphylococci and Staphylococcal Infections, 26th-30th August 2012, Lyon, France

BACKGROUND: MRSA have been a major problem in Irish hospitals for four decades and during this time replacement of predominant nosocomial MRSA clones has occurred several times. Investigating new and emerging MRSA strains and increasing the scientific knowledge base regarding the SCC*mec* elements harboured by these isolates can be beneficial in controlling the spread of MRSA.

METHODOLOGY: As part of a larger investigation of sporadic MRSA strains in Irish hospitals, one distinct isolate recovered in 2006 exhibited a rare *spa* (t878) and multilocus-sequence (ST779) type and was non-typeable using PCR- and DNA microarray-based SCC*mec* typing. Whole-genome sequencing of this isolate (M06/0171) was undertaken using the Illumina Solexa platform and following *de novo* assembly the genetic organisation of a pseudo SCC*mec*-SCC element was elucidated. The structural organisation of the element was confirmed by contig-gap closure using PCR and amplimer sequencing. The novel element was also detected by PCR in an additional 14 t878/ST779-MRSA nosocomial isolates recovered between 2006-2011.

RESULTS & CONCLUSIONS: A ca. 50 kb novel pseudo SCC*mec*-SCC element was identified in 15 t878/ST779-MRSA isolates of which 11 were unrelated epidemiologically. The pseudo SCC*mec*-SCC element, consisted of (i) a pseudo SCC*mec* element (16.2 kb) consisting of *mecA* and a novel *mec* class region, a fusidic acid resistance gene (*fusC*), cadmium and copper resistance genes but lacking *ccr* genes and (ii) a SCC-like element (34.8 kb) with a novel *ccrC* allele, a remnant SCC attachment site, a novel *ccrAB4* allele and a clustered regularly interspaced short palindromic repeat (CRISPR) region. For 16/49 open reading frames (ORFs) identified the highest nucleotide similarity was to ORFs in coagulase-negative staphylococci (CoNS). The identification of this novel pseudo SCC*mec*-SCC element provides further evidence of genetic transfer between *S. aureus* and CoNS and how this contributes to the emergence of novel SCC*mec* elements and MRSA strains. Ongoing surveillance of this MRSA strain is warranted and SCC*mec* typing methods need to be updated to incorporate detection of this novel element.

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Current Staff of the NMRSARL

Dr. Brian O' Connell	Clinical Director
Gráinne Brennan	Chief Medical Scientist
Tanya Fleming	Medical Scientist
Paul Grier	Medical Laboratory Aide

Contact Details

National MRSA Reference Laboratory
St. James's Hospital,
James's Street,
Dublin 8
Ireland

Tel: (+353 1) 410 3662
Fax: (+353 1) 410 3666

Website: www.nmrsarl.ie
Email: mrsarl@stjames.ie

For advice on:

Patient treatment/management

Dr. Brian O'Connell	01 416 2912

Laboratory aspects of MRSA

Gráinne Brennan	01 410 3662

Infection prevention and control

Infection Control Team, SJH	01 416 2961

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