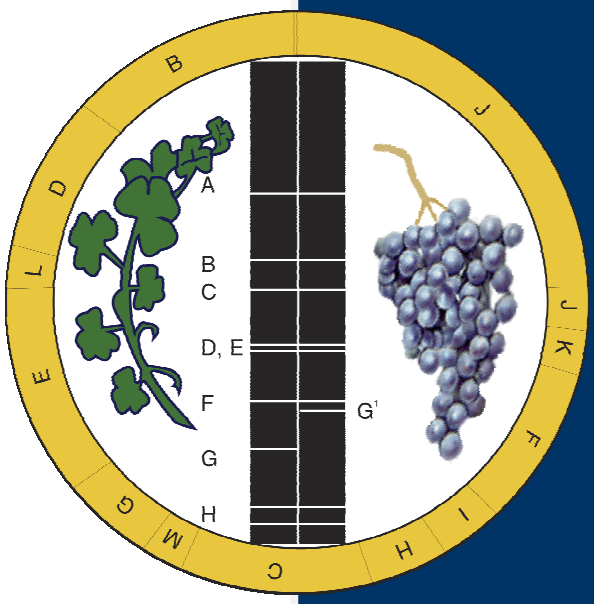
ANNUAL REPORT 2011



National Meticillin-Resistant *Staphylococcus aureus* Reference Laboratory

Table of Contents

Introduction

The National meticillin-resistant *Staphylococcus aureus* Reference Laboratory (NMRSARL) continued to strive to provide provide a high quality service to its users during 2011.

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.

NMRSARL intends to continue to meet the needs of its users into the future and also to enhance the safety of patient care by on-going analysis of strains of MRSA and related staphylococci. In particular, the laboratory is adapting to perform investigations for meticillin-susceptible *S. aureus* (MSSA).

The annual report for 2011 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 2011 were:

- Performing continued surveillance of resistance to glycopeptides (i.e. vancomycin and teicoplanin) and noting continued low levels of resistance- this is re-assuring given the importance of these agents in treating serious infection caused by MRSA.
- Continue to monitor resistance to new antibiotics. The rate of resistance is reassuringly low but there is a need for continued vigilance to detect emergent resistance.
- Involvement in the detection of novel strains of MRSA in Ireland. In particular, NMRSARL contributed to the characterisation of a new strain of MRSA carrying a novel *mecA* which has previously gone undetected (1)[1].
- Involvement in the recognition of the introduction of new strains of community associated MRSA causing hospital outbreaks and assisted in controlling these outbreaks.
- NMRSARL detected the first of a particular MRSA strain that has been associated with pig farming in Europe from a patient in Ireland.
- Introduction of a limited epidemiological typing service and toxin detection service for MSSA.
- 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 quality services.
- The appointment of a joint lecturer with TCD which will strengthen the academic links that already exist between the two institutions.

Clinical Director

Chief Medical Scientist

[1]All references are listed in the bibliography at the end of this report

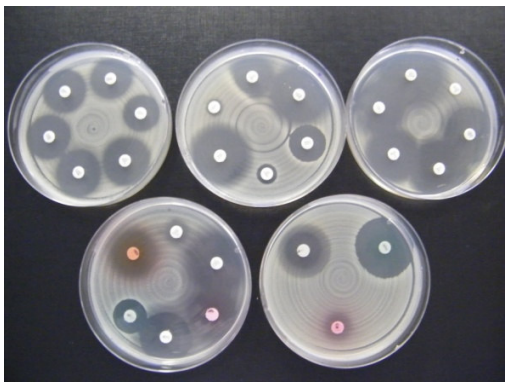
Routine Laboratory Work

European Antimicrobial Resistance Surveillance Network (EARS-Net)

Twenty-nine laboratories throughout Ireland submitted 225 MRSA isolates causing blood-stream infections to NMRSARL during 2011 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 and NMRSARL provides HPSC with data 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.



Fusidic Acid Resistance

Monitoring antimicrobial resistance in MRSA isolates investigated under the EARS-Net has shown that resistance to fusidic acid increased from <10% between 1999 and 2001 to 27% in 2006. While this increase continued through to 2010, in 2011 it fell to 34% (77/225) representing a reduction of 7%.

This overall increased 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.

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 2011, all isolates of MRSA were tested against linezolid and all were susceptible. In addition, 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.

Mupirocin Resistance

Although 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 the later period of 2008 55% of Hi-MupR isolates exhibited this unfamiliar AR pattern (16/29) while 14% (4/29) were AR-PFG 06-01 (2).

In 2011, Hi-MupR was detected in 3.11% (7/225) of MRSA isolates from blood (2.85% in 2010); one of these isolates was recognised as an 'old AR07 strain' while the remaining 85% (6/7 isolates) represented the 'unfamiliar' AR pattern with aminoglycoside resistance but with PFG-01 patterns associated with the AR06 AR type.

Mupirocin is the agent of choice to eradicate nasal colonisation with MRSA. The detection of Hi-MupR in the most common strain circulating in Irish hospitals, is of significant concern, as increasing prevalence will undoubtedly have a major impact on the success of decolonisation, a major strategy in preventing spread.

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

Glycopeptide Resistance

The glycopeptides 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 uses a vancomycin agar screen and an experimental teicoplanin agar screen for preliminary investigation of reduced susceptibility to glycopeptide (3). NMRSARL also tests all EARS-Net MRSA isolates by a commercial MIC system (E-test) for both vancomycin and teicoplanin resistance 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 2011 no EARS-Net isolates exhibited reduced susceptibility to glycopeptides however there were eight reference laboratory isolates selected for further investigation by PAP-AUC. Seven of these were confirmed to exhibit reduced susceptibility to vancomycin and teicoplanin; 85% (6/7) were hGISA while one was a GISA.

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.

Throughout 2011 the AR-PFG 06-01 pattern continued to predominate. This strain is susceptible to most antimicrobials on the AR typing panel resulting in AR typing becoming less useful and PFGE typing being required more extensively.

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.

spa Typing

spa typing recognises mutations or repeat insertion / deletion events that can cause changes in the polymorphic X region of the staphylococcal protein A gene (*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.

In 2011, 125 *S. aureus* isolates were *spa* typed. These included all PVL-positive isolates and other *S. aureus* isolates that were selected based on their AR pattern.

Among the PVL-positive isolates (Figure 1), there were two predominant *spa* types recognised. *spa* type t657 accounted for 38% (17/42) of the isolates while t008 (ST8) accounted for 19% (8/42). These two strains have been widely reported as community-associated strains and are also known as the Bengal Bay strain and USA300 respectively.

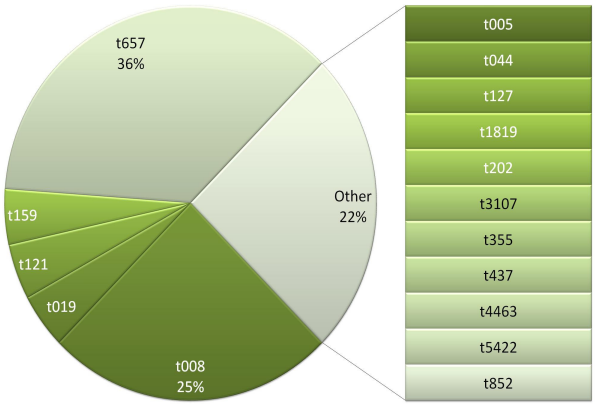


Figure 1. *spa* types exhibited by PVL-positive isolates. *spa* types exhibited by only one isolate are shown to the side of the chart

The Bengal Bay strain was first recognised in Ireland in 2009 among two PVL positive isolates and since then its prevalence has continued to increase. The large increase in the prevalence in 2011 arose as this strain was not only recognised in the Irish community but was also associated with an outbreak in an Irish hospital. In most cases where this strain was recovered, epidemiological data showed that the patient had a connection with the Indian sub-continent.

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 date no such incidents have been reported in Ireland.

PVL- negative isolates selected for *spa* typing include isolates exhibiting AR patterns similar to patterns exhibited by 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 can not be reliably typed by AR typing and required additional investigation (6).

The PVL-negative isolates exhibited much greater diversity. Among these isolates investigated there were 43 different *spa* types (Figure 2) with only six recognised in more than three isolates. There were six novel *spa* types ie *spa* types not previously submitted to the online database.

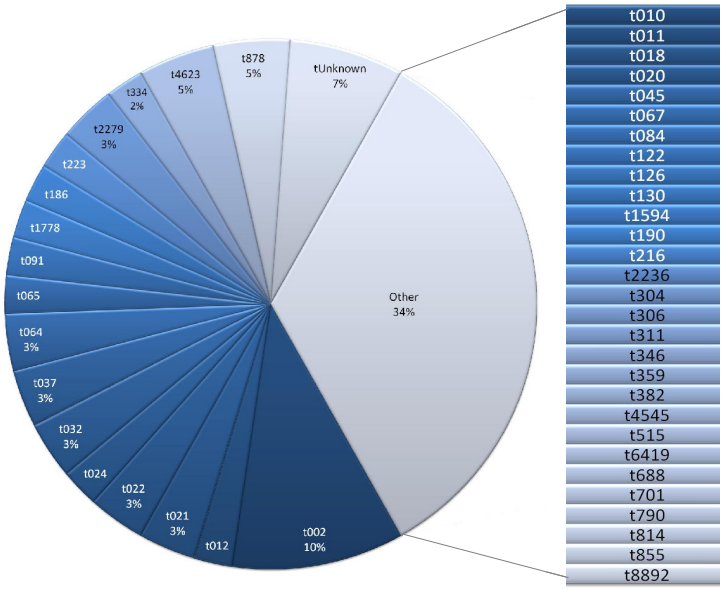


Figure 2. *spa* types exhibited by PVL-negative isolates.
spa types exhibited by only one isolate are shown to the side of the chart

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.

In previous reports, we showed 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. The correlation between AR-PFG, MLST and SCC*mec* types is shown in Figure 3. Isolates with AR-PFG 06-01 exhibited MLST and SCC*mec* type ST22-MRSA-IV similar to UK EMRSA-15 while those with AR-PFG 07-02 were ST36-MRSA-II similar to UK EMRSA-16.

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 due to an increase in the number of unfamiliar strains recovered. These isolates were further investigated by *spa* typing and data for these isolates are included in the data shown in Figures 1 and 2.

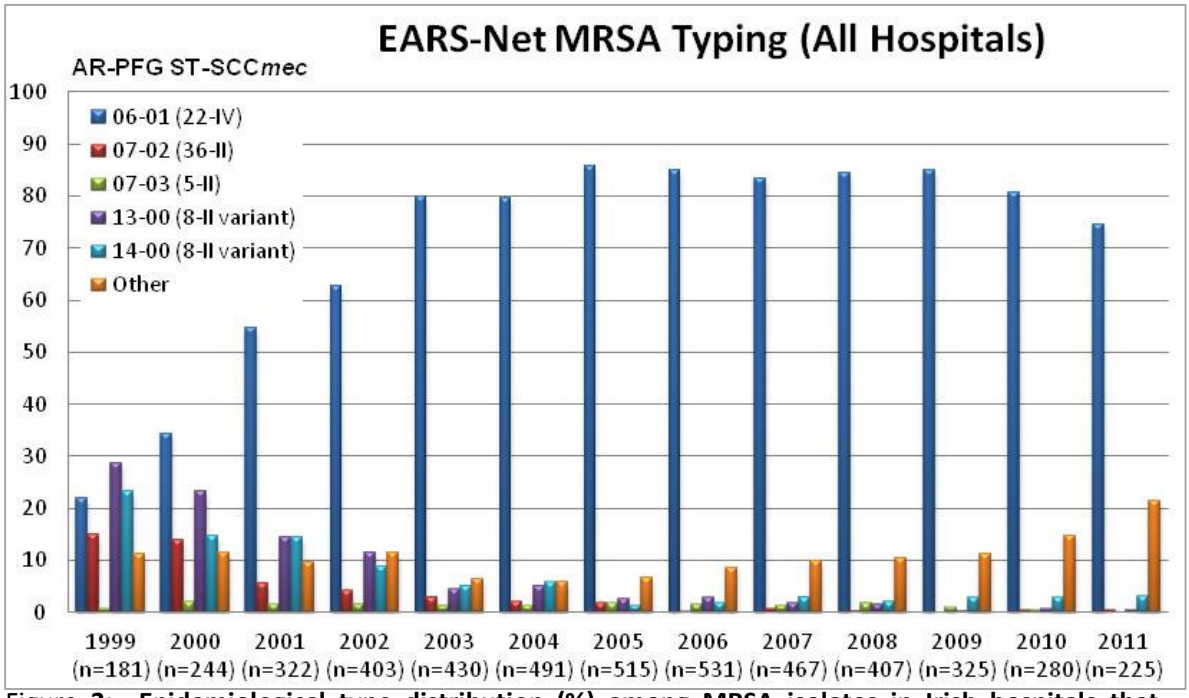


Figure **3: Epidemiological type distribution (%) among MRSA isolates in Irish hospitals that participate in the European Antimicrobial Resistance Surveillance Network (1999 to 2011).**

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) (Ref).

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 2011, 251 isolates of MRSA and meticillin-susceptible *S. aureus* (MSSA) were tested for carriage of PVL on the basis of clinical or epidemiological need. These comprised 29 EARS-Net MRSA isolates, 61 MSSA and 161 MRSA isolates. Five percent of MSSA (3/61) and 24% of MRSA (39/161) were PVL-positive. Additional molecular epidemiology information is available in the *spa* typing section above.

MRSA Associated with Animals

In 2011, NMRSARL continued its collaborative work with veterinary colleagues (8).

The most well-known livestock associated MRSA strain exhibits the genotype ST398. It 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.

During 2011, NMRSARL recognised the first case of this strain in Ireland in an isolate recovered from an elderly patient.

The report of the discovery of the novel mecA variant with less than 70% homology with the mecA gene is of great importance and staff in NMRSARL were involved in the recognition of the first cases of these isolates in Ireland and worldwide (1). While Irish isolates of this strain have been recovered from human patients throughout Europe, this strain has been recovered from cattle and so it is thought to be of bovine origin.

NMRSARL alerted hospital microbiology staff of the difficulties in detecting this strain of MRSA due to the low level oxacillin resistance it exhibits.

Requests for Support and Assistance from Diagnostic Laboratories

During 2011, the routine epidemiological typing service was limited to institutions experiencing acute problems and AR typing and biotyping only were routinely offered. A change in the way in which isolates were selected for further investigation based on the information obtained from those results led to a reduction in the number of isolates investigated by PFGE but an increase in the number of isolates *spa* typed.

The chart below summarises the number of episodes when the NMRSARL has assisted diagnostic laboratories during 2011 along with the number of tests performed on isolates received within the laboratory.

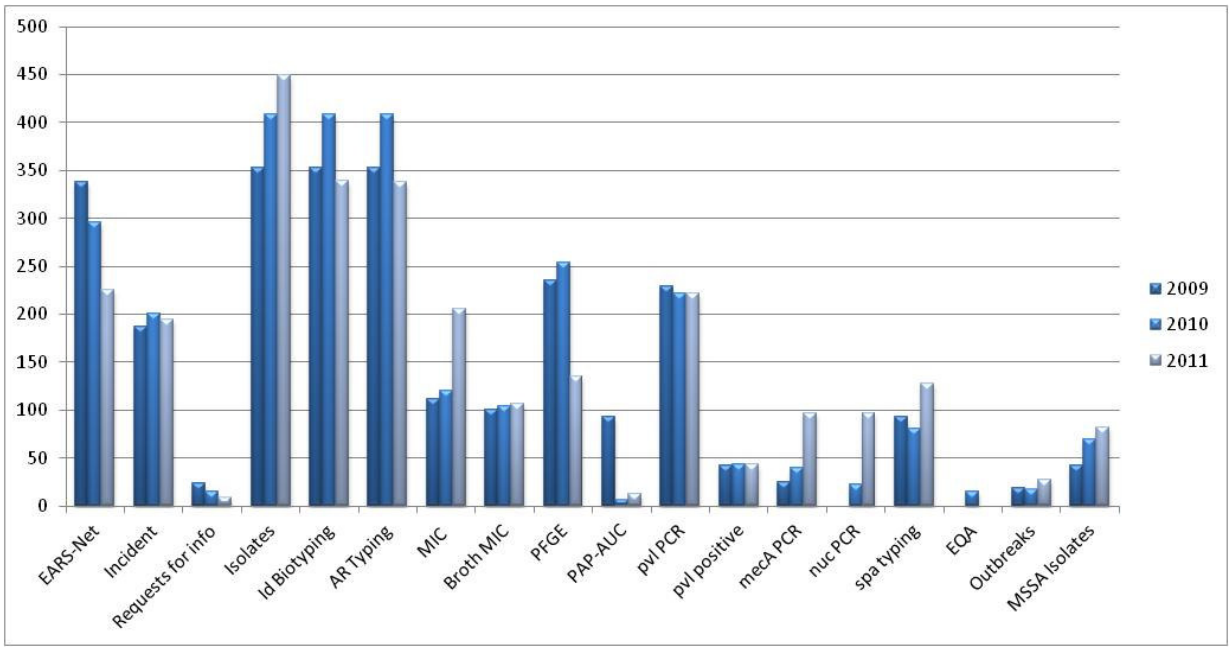


Figure4: The breakdown of incidents, isolates and tests performed during 2011.
pvl, Panton-Valentine leukocidin toxin gene; *mecA*, gene encoding meticillin resistance; *spa*, staphylococcal protein A gene typing; PFGE, pulsed field gel electrophoresis. Id Biotyping, MIC, AR Typing and PFGE is performed all EARS-Net isolates.

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.

Quality Objectives

NMRSARL is committed to providing a quality service and to developing the laboratory in line with the needs and requirements of it users. During 2010, NMRSARL carried out a user satisfaction survey in order to assess user opinions of the service and to ensure that the range of tests provided by the laboratory meet the needs of users. Results from this survey were used in setting Quality objectives for 2011 along with other internal objectives relating to Staff Training and Education, Health and Safety and Waste Management.

Progress report on user related Quality Objectives

1. Introduction of the investigation 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.

2. Introduction of real-time PCR through the development of a triplex assay for the simultaneous detection of PVL, *mecA* and *nuc*

An undergraduate student from The Dublin Institute of Technology, Kevin Street validated this assay as part of her final year project. The assay has been in routine use since September 2011 and has been expanded to include detection of the novel *mecA* in Ireland.

3. Participation in the EARS-Net *spa* typing project

Isolates were collected through the first half of 2011 and analysis of results of the isolates is underway in Europe. NMRSARL would like to thank staff in all the Irish laboratories who submitted isolates and data for this study.

Resources

Staff

In January 2011 Ms. Gráinne Brennan was appointed as Chief Medical Scientist of the laboratory. Other staff employed at NMRSARL included one medical scientist (Ms. Emma Gibbons), one molecular microbiologist (Dr. Pamela Morgan) and one medical laboratory aide (Mr. Paul Grier).

During 2011 one member of staff went on a career break while another went on maternity leave and these vacant posts were filled by Ms. Tanya Fleming and Mr. James Kerins.

Dr. Anna Shore was appointed as a Lecturer in Applied and Translational Microbiology. This role is a joint appointment between the School of Dental Science and School of Medicine and involves 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.

Premises and Equipment

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.

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

Funding of €281k was allocated to NMRSARL for the year 2011. Despite this reduction on previous years NMRSARL stayed within budget for the year.

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 microbiology students in the Moyne Institute, TCD 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, computer skills, management skills, the quality management system and journal clubs organized within St. James's Hospital.

One member of staff began an MSc in Clinical Laboratory Science through DIT, Kevin Street. The laboratory also facilitated an undergraduate student from the DIT, Kevin Street for the completion of her final year project. Two students completed 2 month work experience placement programmes.

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:

- MLVA Practical Workshop, RIVM, Amsterdam
- Staphylococcal Reference Laboratories Working Group, Cambridge, UK
- Focus on Infection, TCD
- Occupational First Aid, St. John's Ambulance
- Academy of Medical Laboratory Science, Microbiology Advisory Body Meetings
- Academy of Medical Laboratory Science, Quality Advisory Body Meetings

2011 also saw the Third National MRSA Reference Laboratory Scientific Meeting held in the Royal College of Physicians, Ireland where a panel of national and international experts addressed delegates on a wide range of topics including the epidemiology, screening and treatment of MRSA along with community-associated MRSA.

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 (SCCmecXI) 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.

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 genotypes of high level mupirocin resistant (Hi-MupR) MRSA isolates. In particular current research is focussing on Hi-MupR-conferring plasmids in isolates exhibiting AR type AR06 and the 'unfamiliar' AR pattern with aminoglycoside resistance. These isolates exhibit PFG 01 patterns associated with the AR06 AR type.

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

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

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

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

7. 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.

8. 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

Collaborative work with Professor Richard Goering, Creighton University, Nebraska, USA proved fruitful in providing a potentially discriminatory method for distinguishing among AR-PFG 06-01 MRSA.

Discussions with staff from the Scottish, Danish and UK Reference Laboratories to organize an external quality assessment (EQA) programme between the four centers continue.

Publications by staff of the NMRSARL

Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe.

Emerg Infect Dis. 2011 Mar; 17(3):502-5.

van Cleef BA, Monnet DL, Voss A, Krziwanek K, Allerberger F, Struelens M, Zemlickova H, Skov RL, Vuopio-Varkila J, Cuny C, Friedrich AW, Spiliopoulou I, Pászti J, Hardardottir H, Rossney A, Pan A, Pantosti A, Borg M, Grundmann H, Mueller-Premru M, Olsson-Liljequist B, Widmer A, Harbarth S, Schweiger A, Unal S, Kluytmans JA.

Abstract

To estimate the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from humans that were sequence type (ST) 398, we surveyed 24 laboratories in 17 countries in Europe in 2007. Livestock-associated MRSA ST398 accounted for only a small proportion of MRSA isolates from humans; most were from the Netherlands, Belgium, Denmark, and Austria.

A study of the prevalence of methicillin-resistant *Staphylococcus aureus* in pigs and in personnel involved in the pig industry in Ireland.

Vet J. 2011 Nov; 190 (2):255-9.

Horgan M, Abbott Y, Lawlor PG, Rossney A, Coffey A, Fitzgerald GF, McAuliffe O, Paul Ross R.

Abstract

To evaluate the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the pig population in Ireland, nasal swabbing was employed in three abattoirs to screen 440 pigs from 41 geographically distributed farms. One hundred individuals involved in the pig industry were also nasally screened. No MRSA isolates were recovered from the pigs and only two of the humans tested were identified as MRSA carriers. Importantly, MRSA was not obtained from pig producers, veterinarians or abattoir employees, but was isolated from individuals working in the wider pig industry. Multi-locus sequence typing revealed that these isolates belonged to sequence types (ST) ST22 and ST1307; the latter is a previously unreported single locus variant of ST5. Five dust samples from each of the three slaughterhouses were culture-negative for MRSA. These results indicate that porcine colonisation by MRSA, and in particular the animal-related strain MRSA-ST398, was not common in Ireland during the period of study.

Characterization of a novel arginine catabolic mobile element (ACME) and staphylococcal chromosomal cassette *mec* compositeisland with significant homology to *Staphylococcus epidermidis* ACME type II in methicillin-resistant *Staphylococcus aureus* genotype ST22-MRSA-IV.

Antimicrob Agents Chemother. 2011 May; 55(5):1896-905.

Shore AC, Rossney AS, Brennan OM, Kinnevey PM, Humphreys H, Sullivan DJ, Goering RV, Ehricht R, Monecke S, Coleman DC.

Abstract

The arginine catabolic mobile element (ACME) is prevalent among methicillin-resistant *Staphylococcus aureus* (MRSA) isolates of sequence type 8 (ST8) and staphylococcal chromosomal cassette *mec* (SCC*mec*) type IVa (USA300) (ST8-MRSA-IVa isolates), and evidence suggests that ACME enhances the ability of ST8-MRSA-IVa to grow and survive on its host. ACME has been identified in a small number of isolates belonging to other MRSA clones but is widespread among coagulase-negative staphylococci (CoNS). This study reports the first description of ACME in two distinct strains of the pandemic ST22-MRSA-IV clone. A total of 238 MRSA isolates recovered in Ireland between 1971 and 2008 were investigated for ACME using a DNA microarray. Twenty-three isolates (9.7%) were ACME positive, and all were either MRSA genotype ST8-MRSA-IVa (7/23, 30%) or MRSA genotype ST22-MRSA-IV (16/23, 70%). Whole-genome sequencing and comprehensive molecular characterization revealed the presence of a novel 46-kb ACME and staphylococcal chromosomal cassette *mec* (SCC*mec*) composite island (ACME/SCC*mec*-CI) in ST22-MRSA-IVh isolates (n=15). This ACME/SCC*mec*-CI consists of a 12-kb DNA region previously identified in ACME type II in S. epidermidis ATCC 12228, a truncated copy of the J1 region of SCC*mec* type I, and a complete SCC*mec* type IVh element. The composite island has a novel genetic organization, with ACME located within orfX and SCC*mec* located downstream of ACME. One PVL locus-positive ST22-MRSA-IVa isolate carried ACME located downstream of SCC*mec* type IVa, as previously described in ST8-MRSA-IVa. These results suggest that ACME has been acquired by ST22-MRSA-IV on two independent occasions. At least one of these instances may have involved horizontal transfer and recombination events between MRSA and CoNS. The presence of ACME may enhance dissemination of ST22-MRSA-IV, an already successful MRSA clone.

Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecI, mecR1, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*.

Antimicrob Agents Chemother. 2011 Aug; 55(8):3765-73.

Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, Ehricht R, Coleman DC.

Abstract

Methicillin resistance in staphylococci is mediated by penicillin binding protein 2a (PBP 2a), encoded by mecA on mobile staphylococcal cassette chromosome *mec* (SCC*mec*) elements. In this study, two clonal complex 130 (CC130) methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from patients in Irish hospitals were identified that were phenotypically PBP 2a positive but lacked *mecA* by conventional PCR and by DNA microarray screening. The isolates were identified as methicillin-susceptible *S. aureus* using the GeneXpert real-time PCR assay. Whole-genome sequencing of one isolate (M10/0061) revealed a 30-kb SCC*mec* element encoding a class E *mec* complex with highly divergent *blaZ*-*mec*A-*mec*R1-*mec*I, a type 8 cassette chromosome recombinase (*ccr*) complex consisting of *ccr*A1-*ccr*B3, an arsenic resistance operon, and flanking direct repeats (DRs). The SCC*mec* element was almost identical to that of SCC*mec* type XI (SCC*mec* XI) identified by the Sanger Institute in sequence type 425 bovine MRSA strain LGA251 listed on the website of the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements. The open reading frames (ORFs) identified within SCC*mec* XI of M10/0061 exhibited 21 to 93% amino acid identity to ORFs in GenBank. A third DR was identified ca. 3 kb downstream of SCC*mec* XI, indicating the presence of a possible SCC remnant. SCC*mec* XI was also identified in the second CC130 MRSA isolate by PCR and sequencing. The CC130 MRSA isolates may be of animal origin as previously reported CC130 S. aureus strains were predominantly from bovine sources. The highly divergent nature of SCC*mec* XI relative to other SCC*mec* elements indicates that it may have originated in another taxon.

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.

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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 (SCCmec) 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 multiantibiotic 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.

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