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INTRODUCTION

The primary role of the National meticillin-resistant *Staphylococcus aureus* Reference Laboratory (NMRSARL) is to assist routine microbiology hospitals in the correct identification and control of MRSA using specialized molecular and epidemiological typing techniques.

During 2013 the NMRSARL continued to provide a high quality service to its users and this annual report for 2014 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 2014 were:

- Achieving accreditation in the ISO15189 standards under INAB
- Continuing to perform 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;
- Continuing to monitor resistance to new antibiotics and noting continued lack of resistance. 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.

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 (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.
**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 2014 along with the number of tests performed on isolates received within the laboratory.

Briefly, the declining rates of MRSA in Irish hospitals continued in 2014 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 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.

During the year the NMRSARL also assisted several users investigating issues arising with coagulase negative Staphylococci and while the NMRSARL’s knowledge is limited with regards to CNS, some techniques used when investigating MRSA may also be applied to other Staphylococcal species.

**European Antimicrobial Resistance Surveillance Network (EARS-Net)**

Twenty-six laboratories throughout Ireland submitted 185 MRSA isolates causing bloodstream infections to NMRSARL during 2014 for monitoring of epidemiological types and resistance to clinically significant antimicrobials. Clinical data on these isolates is submitted to the Health Protection Surveillance Centre (HPSC) under the EARS-Net project, a European initiative that, in Ireland and this data generated from the laboratory work in the NMRSARL provides HPSC with information on rates of resistance to clinically useful antibiotics.

All isolates undergo Antibiogram-Resistogram (AR) typing performed in accordance with EUCAST methodology using a panel of 23 antimicrobial agents. Where interpretive criteria are not available from EUCAST, criteria set by the Clinical and Laboratory Standards Institute (CLSI) or developed in the NMRSARL is used. 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 2014 compared with that of the previous years. \textit{pvl}, Panton-Valentine leukocidin toxin gene; \textit{mecA}, gene encoding meticillin resistance; \textit{mecC}, gene encoding meticillin resistance \textit{spa}, staphylococcal protein A gene typing; PFGE, pulsed field gel electrophoresis. Id Biotyping, MIC, AR Typing is on performed all EARS-Net isolates.
MOLECULAR EPIDEMIOLOGICAL TYPING OF MRSA

Typing methods for discriminating different bacterial isolates of the same species are essential epidemiological tools in infection prevention and control. Traditional typing systems based on phenotypic characteristics such as antibiogram, have been used for many years. However, the clonality of the predominant strain of MRSA in Ireland has meant that these traditional typing methods fail to provide sufficient discrimination of isolates in outbreak situations (1). 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 (2).

In recent years NMRSARL has increased the number of isolates investigated by *spa* typing and in 2014, 418 isolates representing a 50% increase on the number of isolates investigated in 2013. Isolates investigated by *spa* typing included 41 isolates recovered from blood culture specimens, 91 MSSA isolates, 80 PVL-positive isolates, X isolates involved in outbreaks and X isolates exhibiting unusual phenotypic characteristics.

Among the isolates there were 167 different *spa* types recognised. As in previous years, there was great diversity seen among the MSSA isolates (67 types among 91 isolates) in comparison to the MRSA (100 types among 327 isolates). BURP analysis clustered all the isolates into 17 different clonal clusters however 75% of the isolates were clustered into just five groups; ST22 (33%), ST30, ST5, ST8 and ST1, each of which includes both MRSA and MSSA isolates and PVL- positive and PVL-negative isolates.
Among the MRSA isolates exhibiting unfamiliar AR patterns the most frequently occurring spa type was t127, associated with ST1 (26%, 40/152). The large increase in this strain is due primarily to an outbreak in caused by this strain in one hospital however 27% of the total t127 received (11/40) were not associated with this outbreak and were recovered elsewhere in Ireland both in hospitals and from patients attending GPs. While some isolates of this type exhibit multi antibiotic resistance (aminoglycosides, mupirocin, tetracycline, fusidic acid, ciprofloxacin and/or erythromycin) others exhibit a susceptible profile with resistance only to ampicillin.

While the majority of outbreaks investigated by spa typing throughout the year were due to ST22 associated strains, other strains did cause problems in units in Irish hospital and these included t786 (ST88) and t311 (ST5).

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 virulence than the ST22 strains and so close monitoring is required in order to control the spread of these strains in the hospital setting.

**ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND**

Like Europe, ST22-MRSA-IV is the pandemic clone in Ireland and, in 2014, was associated with 75% 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 (3).

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 (3).

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) (3).

In Ireland in 2014 the most frequently occurring spa type among the ST22 isolates was t032 however a further 40 other spa types associated with ST22, including among others t022, t515, t223, t2945 and t020 were also recognised.
PVL-POSITIVE S. AUREUS IN IRELAND

During 2013 staff in the NMRSARL and collaborators in the Dublin Dental University Hospital published work characterizing all PVL positive S. aureus isolates submitted to the laboratory between 2002 and 2011 (4). DNA microarray analysis was used to detect 334 S. aureus genes including antimicrobial resistance and virulence genes and capsule types while also assigning isolates to a multilocus sequence type clonal complex and sequence type.

In total 229 isolates were included in the study and it was found that the epidemiology of pvl-positive S. aureus is changing. There were 16 different genotypes recognized among 190 MRSA isolates and five genotypes among 39 MSSA isolates. Predominant genotypes included CC/ST8-MRSA-IV, CC/ST30-MRSA-IV, CC/ST80-MRSA-IV, CC1/ST772-MRSA-V, CC30-MSSA, CC22-MSSA, and CC121-MSSA. Using epidemiological data it was found that some strains had been imported into Ireland on several different occasions and that 70% of isolates for which patient data was available were from the community including six family clusters of pvl-positive MRSA.

In 2014, 527 S. aureus isolates were tested for carriage of the lukS-PV and lukF-PV genes encoding for PVL and of these 15% were PVL-positive. These isolates were selected based on clinical presentation or phenotypic epidemiological typing data and included 35 EARS-Net MRSA isolates, 220 meticillin-susceptible S. aureus and 307 MRSA isolates. Seven percent of MSSA (17/220) and 21% of MRSA (64/307) were PVL-positive.

A review of clinical information submitted with these isolates showed that skin and soft tissue infection was the predominant clinical presentation along with links to other countries through ethnicity, travel or family contacts.

spa type t008, which is associated with ST8, accounted for the highest spa type among PVL-positive isolates. Also associated with USA300, this spa type has been recognised among Irish PVL-positive isolates since 2007 however this is the highest number of isolates of this spa type recovered in a given year (32.5%, 26/80). 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 spa types recognised among the PVL-positive isolates were spa type t852, t019 and t657, associated with ST22, ST30 and ST1 respectively. These strains have been previously recognised in Ireland and while there is a reduction in the number of t852 and t657 isolates, t019 has increased and is associated with 10% of the PVL-positive isolates (8/80).
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.

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 and in 2014 47.5% (88/185) of all EARS-Net isolates exhibited 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. There are several reported genes associated with fusidic acid resistance in S. aureus and while resistance among ST22-MRSA-IV is predominantly related to mutations in the fusA gene, resistance among other strains is primarily related to acquisition of the fusB or fusC genes both of which have also been reported among CNS strains.

Glycopeptide Resistance

Vancomycin and teicoplanin remain the mainstay for the treatment of serious infection caused by MRSA and there has been increasing concern about the development of resistance to these agents in recent years.

All isolates are screened 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 2014, while no EARS-Net isolates exhibited reduced susceptibility to glycopeptides, a number of isolates from other sources were submitted to the laboratory for glycopeptide investigation and six of these were confirmed as hGISA using PAP-AUC determination.
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. More recently resistance has been observed in an unfamiliar AR pattern exhibiting mupirocin resistance along with resistance to the aminoglycosides gentamicin, kanamycin and tobramycin and which molecular typing has shown is associated with ST22.

During 2014, mupirocin resistant was recognized among four percent (8/185) of EARS-Net isolates which included both ST22 isolates, older strains of Irish MRSA and other possibly community associated strains. Collaborative work involving NMRSARL and the DDUH includes investigating the Hi-MupR–conferring plasmids in these isolates.

Linezolid, Quinupristin/Dalfopristin, Ceftaroline, Daptomycin and Tigecycline

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 2014 a collection of 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, ceftaroline and tigecycline by disk diffusion. While all isolates were susceptible.

In addition to the EARS-net isolates further isolates were investigated for resistance to daptomycin and 3 were found to exhibit reduced susceptibility. This resistance was confirmed in another research facility by broth microdilution.

All isolates submitted to the laboratory were also tested against linezolid and while no MRSA isolates were found to be resistant, the NMRSARL assisted users in the investigation of linezolid resistance in a Staphylococcus epidermidis which has been associated with an outbreak in their hospital.

In general resistance to linezolid among Staphylococci is unusual and we are working with our research colleagues in investigating the the plasmids carrying cfr and other mechanisms of resistance (5).
EDUCATION

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;

- Rapid Next Generation sequencing workshop
- Journal clubs
- International symposium on Staphylococci & Staphylococcal Infections
- 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 started on the MSc in Clinical Laboratory Science through DIT, Kevin Street while another member of staff started a research PhD on the characterisation of sporadically occurring MRSA in Ireland through the Dublin Dental Hospital, TCD.

Research Highlights

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

These include a close collaboration with Prof. David Coleman characterizing emerging strains of MRSA in Ireland, including PVL-positive strains, using different technology (DNA microarray and whole genome sequencing) and determining the role this technology plays in investigation of outbreaks. Along with MRSA, additional work has been carried out on coagulase negative Staphylococci and MSSA isolates recovered in Irish hospitals.

The laboratory also collaborates with Dr. Finola Leonard, UCD monitoring strains of MRSA which in animals which have been reported to cause infections in humans. In particular this involves the ST398 MRSA which, although reported throughout Europe since the 2000s, was only first recognised in Ireland in 2010.

The laboratory also participates in the European Staphylococcal Reference Laboratory Working Group. This group completed a study in 2006 characterising a collection of isolates recovered in Europe. A second study was carried out in 2011 and this work was published at the end of 2014. Further publications involving these isolates are in progress.

Below are abstracts resulting from these very successful collaborations which have been published throughout the year or posters that were presented at international conferences.
PUBLICATIONS


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


Abstract

Clonal replacement of predominant nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) strains has occurred several times in Ireland during the last 4 decades. However, little is known about sporadically occurring MRSA in Irish hospitals or in other countries. Eighty-eight representative *pvl*-negative sporadic MRSA isolates recovered in Irish hospitals between 2000 and 2012 were investigated. These yielded unusual pulsed-field gel electrophoresis and antibiogram-resistogram typing patterns distinct from those of the predominant nosocomial MRSA clone, ST22-MRSA-IV, during the study period. Isolates were characterized by spa typing and DNA microarray profiling for multilocus sequence type (MLST) clonal complex (CC) and/or sequence type (ST) and SCC*mec* type assignment, as well as for detection of virulence and antimicrobial resistance genes. Conventional PCR-based SCC*mec* subtyping was undertaken when necessary. Extensive diversity was detected, including 38 *spa* types, 13 MLST-CCs (including 18 STs among 62 isolates assigned to STs), and 25 SCC*mec* types (including 2 possible novel SCC*mec* elements and 7 possible novel SCC*mec* subtypes). Fifty-four MLST-*spa*-SCC*mec* type combinations were identified. Overall, 68.5% of isolates were assigned to nosocomial lineages, with ST8-t190-MRSA-IIId/IIE±SCCM1 predominating (17.4%), followed by CC779/ST779-t878-MRSA-ψSCCmec-SCC-SCCCRISPR (7.6%) and CC22/ST22-t032-MRSA-IVh (5.4%). Community-associated clones, including CC1-t127/t386/t2279-MRSA-IV, CC59-t216-MRSA-V, CC8-t008-MRSA-IVa, and CC5-t002/t242-MRSA-IV/V, and putative animal-associated clones, including CC130-t12399-MRSA-XI, ST8-t064-MRSA-IVa, ST398-t011-MRSA-IVa, and CC6-t701-MRSA-V, were also identified. In total, 53.3% and 47.8% of isolates harbored genes for resistance to two or more classes of antimicrobial agents and two or more mobile genetic element-encoded virulence-associated factors, respectively. Effective ongoing surveillance of sporadic nosocomial MRSA is warranted for early detection of emerging clones and reservoirs of virulence, resistance, and SCC*mec* genes.
Panton-Valentine leukocidin-positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant *S. aureus* clones, and increasing multiresistance

Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC.

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**Abstract**

There has been a worldwide increase in community-associated (CA) methicillin-resistant *Staphylococcus aureus* (MRSA) infections. CA-MRSA isolates commonly produce the Panton-Valentine leukocidin toxin encoded by the *pvl* genes *lukF-PV* and *lukS-PV*. This study investigated the clinical and molecular epidemiologies of *pvl*-positive MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates identified by the Irish National MRSA Reference Laboratory (NMRSARL) between 2002 and 2011. All *pvl*-positive MRSA (*n*=190) and MSSA (*n*=39) isolates underwent antibiogram-resistogram typing, *spa* typing, and DNA microarray profiling for multilocus sequence type, clonal complex (CC) and/or sequence type (ST), staphylococcal cassette chromosome *mec* type assignment, and virulence and resistance gene detection. Where available, patient demographics and clinical data were analyzed. The prevalence of *pvl*-positive MRSA increased from 0.2% to 8.8%, and that of *pvl*-positive MSSA decreased from 20% to 2.5% during the study period. The *pvl*-positive MRSA and MSSA isolates belonged to 16 and 5 genotypes, respectively, with CC/ST8-MRSA-IV, CC/ST30-MRSA-IV, CC/ST80-MRSA-IV, CC1/ST772-MRSA-V, CC30-MSSA, CC22-MSSA, and CC121-MSSA predominating. Temporal shifts in the predominant *pvl*-positive MRSA genotypes and a 6-fold increase in multiresistant *pvl*-positive MRSA genotypes occurred during the study period. An analysis of patient data indicated that *pvl*-positive *S. aureus* strains, especially MRSA strains, had been imported into Ireland several times. Two hospital and six family clusters of *pvl*-positive MRSA were identified, and 70% of the patient isolates for which information was available were from patients in the community. This study highlights the increased burden and changing molecular epidemiology of *pvl*-positive *S. aureus* in Ireland over the last decade and the contribution of international travel to the influx of genetically diverse *pvl*-positive *S. aureus* isolates into Ireland.
A longitudinal study of *Staphylococcus aureus* colonization in pigs in Ireland


Abstract

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in livestock has refocused attention on *S. aureus* colonization and transmission in pigs. This study investigated the effect of the *S. aureus* colonization status of a sow on the colonization status of her piglets, and whether pigs carry the same strain of *S. aureus* throughout production. Nasal swabs were collected from the piglets of six healthy sows two days after birth and two days before and two days after they were moved into each production stage. The average prevalence of *S. aureus* colonization varied between 26% and 73%. The odds of being *S. aureus* positive were almost 12 times higher for piglets born to nasal-positive sows than for those born to nasal-negative sows, and three times higher again for piglets born to sows that were both nasal- and vaginal-positive. Isolates recovered from piglets immediately after birth were indistinguishable from those of the dam as determined by phenotypic and molecular typing, including microarray analysis and optical mapping. All isolates belonged to clonal complex 9 and the majority exhibited a novel spa type, t10449. The findings show that the *S. aureus* colonization status of the sow influences the colonization status of her piglets in the early production stages but strains carried by pigs change over time. Multiresistant *S. aureus* was detected, in particular post-weaning. Results suggest that sow status and management practices, including mixing of pigs and antimicrobial usage at weaning, should be considered when implementing control measures for *S. aureus* on a farm.
The dynamic changes of dominant clones of Staphylococcus aureus causing bloodstream infections in the European region: Results of a second structured survey


Euro Surveill. 2014;19(49):pii=20987. Available online:
http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20987

Abstract

*Staphylococcus aureus* is one of the most important human pathogens and meticillin-resistant *S. aureus* (MRSA) presents a major cause of healthcare- and community-acquired infections. This study investigated the spatial and temporal changes of *S. aureus* causing bacteraemia in Europe over a five-year interval and explored the possibility of integrating pathogen-based typing data with epidemiological and clinical information at a European level. Between January 2011 and July 2011, 350 laboratories serving 453 hospitals in 25 countries collected 3,753 isolates (meticillin-sensitive *S. aureus* (MSSA) and MRSA) from patients with *S. aureus* bloodstream infections. All isolates were sent to the national staphylococcal reference laboratories and characterised by quality-controlled *spa* typing. Data were uploaded to an interactive web-based mapping tool. A wide geographical distribution of *spa* types was found, with some prevalent in all European countries. MSSA was more diverse than MRSA. MRSA differed considerably between countries with major international clones expanding or receding when compared to a 2006 survey. We provide evidence that a network approach of decentralised typing and visualisation of aggregated data using an interactive mapping tool can provide important information on the dynamics of *S. aureus* populations such as early signalling of emerging strains, cross-border spread and importation by travel.
POSTERS

Molecular characterisation of livestock-associated ST398 MRSA recovered from humans and animals in Ireland reveals the recent importation and spread of multiple strains

Gráinne I. Brennan, Anna C. Shore, Finola Leonard, Brian O’Connell and David C. Coleman

Posters presented at International Symposium on Staphylococci and Staphylococcal Infections, Chicago, August 2014.

Livestock-associated CC398 methicillin-resistant *Staphylococcus aureus* (MRSA) is widely reported throughout Europe and since 2010 has been identified among animals and humans in Ireland. All CC398 MRSA recovered in Ireland (*n* = 22) were characterized in this study using antimicrobial susceptibility testing, spa typing, DNA microarray profiling and PCR detection of additional antimicrobial resistance genes. CC398 MRSA from a skin sample from a patient with a family member working with pigs abroad, and nasal samples from a nursing-home patient with no animal contact were assigned to SCCmec IV and V and spa types t034 and t011, respectively, and lacked immune evasion complex (IEC) genes. Additional isolates from a foal umbilical abscess and nasal samples from an attending veterinarian were assigned to SCCmec IV and spa type t011, and harbored IEC genes *sak, chp* and *scn*. The remaining CC398 MRSA isolates were from (i) a pig joint abscess, (ii) nasal swabs from other pigs on the same farm (recently restocked with gilts from Germany), (iii) nasal swabs of pigs on a second farm (restocked with pigs from the first farm) and (iv) nasal swabs of workers on these farms. These isolates were assigned to SCCmec V and spa types t011 and t034 and lacked IEC genes. All isolates carried multiple resistance genes including *fexA* (23%), *tet(L)/(K)/(M)* (27/60/91%), *spc* (64%), *aacA-aphD* (23%), *erm(A)/(B)/(C)* (63/27/4.5%) and *dfrG/K* (30/40%). These findings reveal independent introductions of distinct CC398 MRSA strains into Ireland and their zoonotic spread. Increased surveillance is warranted to prevent further importation and spread.
**cfr-mediated linezolid resistance among hospital isolates of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* in Ireland**

Alexandros Lazaris, David C. Coleman, Peter M. Kinnevey, Gráinne I. Brennan, Orla M. Brennan, Angela M. Kearns, Bruno Pichon, Brian O’Connell, Anna C. Shore

**Poster presented at International Symposium on Staphylococci and Staphylococcal Infections, Chicago, August 2014**

Linezolid is often the drug of last resort to treat methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) infections. Linezolid resistance is mediated by mutations within rRNA genes and/or acquisition of the predominantly plasmid located *cfr* gene conferring resistance to phenicols, lincosamides, oxazolidinones, pleuromutulins and streptogramin A compounds (PhLOPS A). In 2010 we reported the characterization of a *cfr*-positive ST8-MRSA-IVa/USA300 isolate from Ireland. Between 2009 and 2014, the Irish National MRSA Reference Laboratory identified a further 22 linezolid-resistant staphylococci from patients in Irish hospitals, mostly during 2013/2014. The present study investigated the mechanism(s) of linezolid resistance among these isolates including one methicillin-susceptible *S. aureus* (MSSA), four MRSA (from two different hospitals) and 17 MRSE (from one hospital). The linezolid MICs for the isolates ranged from 8 to >256 mg/L and all except the MSSA carried *cfr* and exhibited the PhLOPS A phenotype. Mutations within 23S rRNA alleles previously shown to mediate linezolid resistance were identified in the MSSA. The isolates were assigned to CC9-MSSA, CC22-MRSA-IV (spa type t032) and ST2-MRSE-III & Hg by MLST and/or DNA microarray profiling. Whole-genome sequencing and plasmid analysis revealed the presence of conjugative *cfr* & *fexA* plasmids among the MRSA and MRSE. The presence of conjugative *cfr* plasmids in these isolates, particularly among pandemic CC22-MRSA-IV, is worrying and highlights the ability of *cfr* to spread among staphylococci and to complicate treatment options. Prudent management of linezolid use is warranted to prevent linezolid resistance becoming more widespread.
An investigation of ST22-MRSA-IV hospital transmission events using whole-genome sequencing compared with a combination of spa, dru and pulsed-field gel electrophoresis typing and epidemiological data

Peter M. Kinnevey, Anna C. Shore, Micheál MacAogáin, Gráinne I. Brennan, Hilary Humphreys, Brian O’Connell, Thomas R. Rogers, David C. Coleman

Posters presented at International Symposium on Staphylococci and Staphylococcal Infections, Chicago, August 2014.

The ST22-MRSA-IV clone predominates among nosocomial MRSA in several European countries and is endemic in Ireland. ST22-MRSA-IV is highly clonal and tracking its spread is challenging. We previously identified potential transmission events among ST22-MRSA-IV isolates from patients and environmental sites in one Irish hospital during 2007 and 2008 using a combination of spa, dru and pulsed field gel-electrophoresis typing and epidemiological data. In the present study 39 of these ST22-MRSA-IV isolates recovered over six weeks in 2007 underwent whole-genome re-sequencing and single nucleotide variant (SNV) analysis to investigate five transmission events previously identified by molecular epidemiological typing and to determine if other potential transmission events had been missed. Based on previous studies a transmission event was defined as two isolates that differed by ≤ 40 SNVs. Using this criterion, 3/5 previously identified transmission events were supported by whole-genome re-sequencing (6-34 SNVs). Within the remaining two previously identified transmission events, the transmitted isolates differed from source isolates by 54-211 SNVs. Using conventional molecular epidemiological typing, 20/39 isolates were deemed unrelated to any other isolate, compared to 2/39 being unrelated to any other isolate by SNV analysis. Within 741 pairwise comparisons of isolates, 201 (27.1%) differed by ≤40 SNVs (41 (20.4%) from patients only; 55 (27.4%) from environmental sites only; 111 (55.2%) from patient and environmental sites). These results reveal that SNV analysis is significantly more sensitive at identifying ST22-MRSA-IV transmission events than conventional molecular epidemiological typing and indicate a high rate of ST22-MRSA-IV nosocomial transmission, particularly between patients and environmental sites.
Comparative molecular analysis of MRSA and MSSA recovered from bloodstream infections in Irish hospitals

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

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Bloodstream infections (BSIs) caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have declined in Ireland (41.9% in 2006/2007; 24% in 2011) while BSIs caused by methicillin-susceptible *S. aureus* (MSSA) have increased (58.1% in 2006; 72.9% in 2011). This study compared MRSA and MSSA from BSIs over six months in Irish hospitals in 2006/2007 (85 MRSA; 83 MSSA) and 2011 (75 MRSA; 116 MSSA) using spa typing and DNA microarray profiling (Alere, Germany). The 2006/2007 and 2011 MRSA were assigned to seven and six clonal complexes (CCs), respectively, with CC22-MRSA-IV predominating (85.5% 2006/2007; 89% 2011) and to 25 and 21 spa types, respectively, with spa type t032 predominating (45.7% 2006; 46.6% 2011). Greater genetic diversity was detected among the MSSA (2006/2007, 16 CCs and 54 spa types; 2011, 21 CCs and 75 spa types). CC30 (28.9%) and CC45 (18%) MSSA predominated in 2006 and CC45 (17%) and CC7 (12.5%) in 2011. Five common spa types were identified in MRSA (n = 42) and MSSA (n = 16) in 2011 compared to one in 2006/2007 (one MRSA; six MSSA). The intracellular adhesion gene icaC was detected in all 2006/2007 CC22-MRSA-IV but was not detected or yielded ambiguous signals in 50.9% of 2011 CC22-MRSA-IV. This study reveals that despite the decline of MRSA among *S. aureus* BSIs in Ireland, CC22-MRSA-IV continues to predominate. Additionally, temporal changes in the dominant MSSA lineages, an increasing diversity of MSSA lineages and a potential increase in the local emergence of MRSA from MSSA, or *vice versa*, were also identified.
Comparison of SCCmec, antimicrobial resistance genes and clonal lineages of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* recovered from humans and companion animals

Brenda A. McManus, David C. Coleman, Emily C. Deasy, Gráinne I. Brennan, Brian O’Connell, Stefan Monecke, Ralf Ehricht, Yvonne Abbott, Anna C. Shore

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*Staphylococcus epidermidis* and *Staphylococcus haemolyticus* are opportunistic pathogens of humans and animals. This study investigated the genetic relatedness of *S. epidermidis* (SE) and *S. haemolyticus* (SH) isolates from humans (Hu) (29 SE-Hu; 8 SH-Hu,) and companion animals (CpA) (12 SE-CpA; 14 SH-CpA) attending three separate medical centers. All isolates underwent antimicrobial susceptibility testing and DNA microarray profiling (Alere, Germany) to detect antimicrobial resistance and SCCmec genes. Twenty isolates representative of different SCCmec types/subtypes underwent DNA microarray mecA allele identification (Alere). Multilocus sequence typing (MLST) was performed on 48 isolates (14 SE-Hu and 12 SE-CpA; eight SH-Hu and 14 SH-CpA). The majority of isolates exhibited methicillin resistance (MR) and harbored mecA (79% MRSE-Hu; 92% MRSE-CpA; 88% MRSH-Hu; 93% MRSH-CpA). Type IV SCCmec predominated among MRSE (78% MRSE-Hu; 73% MRSE-CpA) and type V SCCmec among MRSH-CpA (46%), whereas the MRSH-Hu isolates were nontypeable. Five distinct mecA alleles were detected; the most prevalent allele (AY786579) was identified in three SE-Hu, three SE-CpA and four SH-Hu isolates. Genes encoding resistance to aminoglycosides (57% Hu; 85% CpA), tetracycline (8% Hu; 54% CpA) and chloramphenicol (0% Hu; 23% CpA) were more prevalent among isolates from CpA. According to MLST, the most prevalent sequence types (STs) (SE-ST2; SH-ST1) were identified among isolates from both humans and companion animals. These findings reveal that *S. epidermidis* and *S. haemolyticus* isolates are likely transmitted between humans and companion animals and highlights the reservoir of antimicrobial resistance genes that exists among these staphylococcal species, particularly from companion animals.
QUALITY MANAGEMENT SYSTEM

In 2014, as part of the LabMed Directorate in St. James’s Hospital, the NMRSARL successfully achieved accreditation with the Irish National Accreditation Board (INAB) under the ISO15189 standards. The quality management system in place within NMRSARL ensures that there are coordinated activities in place in 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 Indicators

Turn-around Times

Verbal reports are issued on urgently requested tests as soon as the results are available. For other isolates received, a printed report is issued with additional tests results reported on subsequent reports.

During 2014, 99% of isolates were reported within the stated turnaround time and where reports exceeded the TAT there were issues relating to mixed culture delayed the report.

Internal Audits

A schedule of audits were completed during 2014 ensuring the quality of pre-examination, examination and post examination processes was maintained. From these audits there were no serious non-conformances identified and any issues raised have been completed.

Service Developments

During 2014, spa typing was used more frequently in outbreak situations replaced PFGE. This sequence-based technique allows greater comparison of isolates typed in the NMRSARL with those recovered at different times in Ireland and those reported from other countries.

Validation of a real-time PCR for the detection of exfoliative toxins etA, etB and etD was completed and this service is now available for users.

The use of electronic reporting was explored in 2014 and it is hoped that this will be available to users in 2015.
RESOURCES

Staff

During 2014 the staff working in the NMRSARL were:

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

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, 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 €258,782. 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.
ACKNOWLEDGEMENTS

NMRSARL would like to thank the departments in SJH who assist with the provision of basic services (Central Pathology Laboratory, Finance, General Support Services, Human Resources and Information Management Services). We especially thank the Microbiology Department for their continued support.

We would also like to thank Professor Hilary Humphreys for his service to the laboratory throughout the year.

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