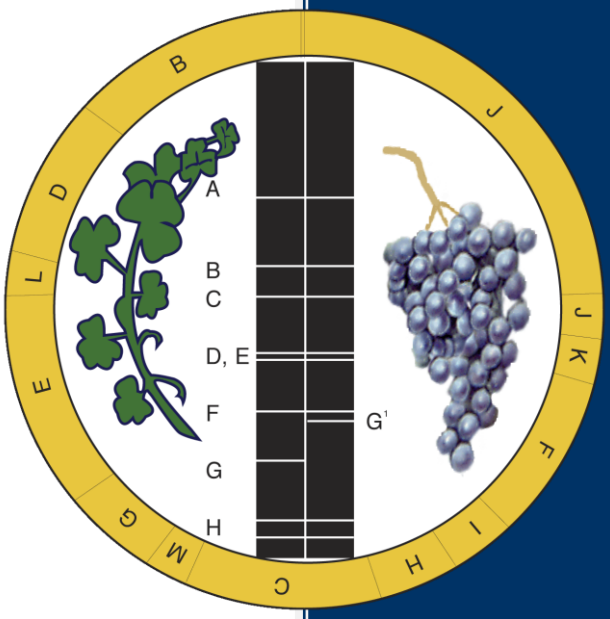
# ANNUAL REPORT 2015




# National Meticillin-Resistant *Staphylococcus aureus* Reference Laboratory

# 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 2014 the NMRSARL continued to provide a high quality service to its users and this annual report 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

Work in the NMRSARL is comprised of two different categories (Figure 1). National surveillance of MRSA in Irish hospitals is done through the European Antimicrobial Resistance Surveillance Network (EARS-Net) and while data is submitted by laboratories directly to the Health Protection Surveillance Centre, MRSA isolates are sent to the NMRSARL for laboratory investigation against a number of antimicrobial agents and to allow monitoring of the circulating strains.

The referral work is comprised of all other isolates submitted to the laboratory for investigation and includes isolates recovered in the community, during outbreak investigations and non- *S. aureus* isolates.

## Reference Laboratory Work

In recent years there has been a steady increase in the number of isolates referred to the NMRSARL for investigation (Figure 1). All requests received are considered incidents and Figure 2 summarises the number of incidents investigated during 2015 along with the number of tests performed on isolates received within the laboratory.

Along with a steady increase in the number of isolates submitted, the complexity of tests has also increased over time. Currently the laboratory performs phenotypic investigation on all isolates submitted however further molecular investigation is performed on over half of the isolates including investigation for PVL toxin (437) or *spa* typing (401).

This change is primarily due to the changing epidemiology of MRSA circulating in Ireland and the limited information that can be obtained from phenotypic investigation of these emerging strains.

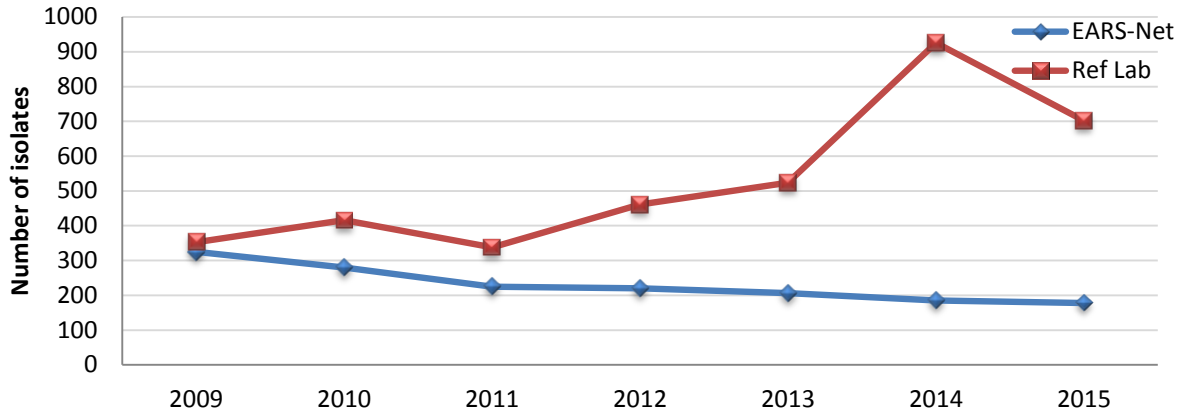
The amount of information obtained from *spa* typing of isolates is invaluable in assisting in the monitoring of strains throughout Ireland and the early detection of emerging strains.

## European Antimicrobial Resistance Surveillance Network

Twenty-six laboratories throughout Ireland submitted 178 MRSA isolates causing blood-stream infections to NMRSARL during 2015 for monitoring of epidemiological types and resistance to clinically significant antimicrobials. Clinical data on these isolates is submitted to the 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.

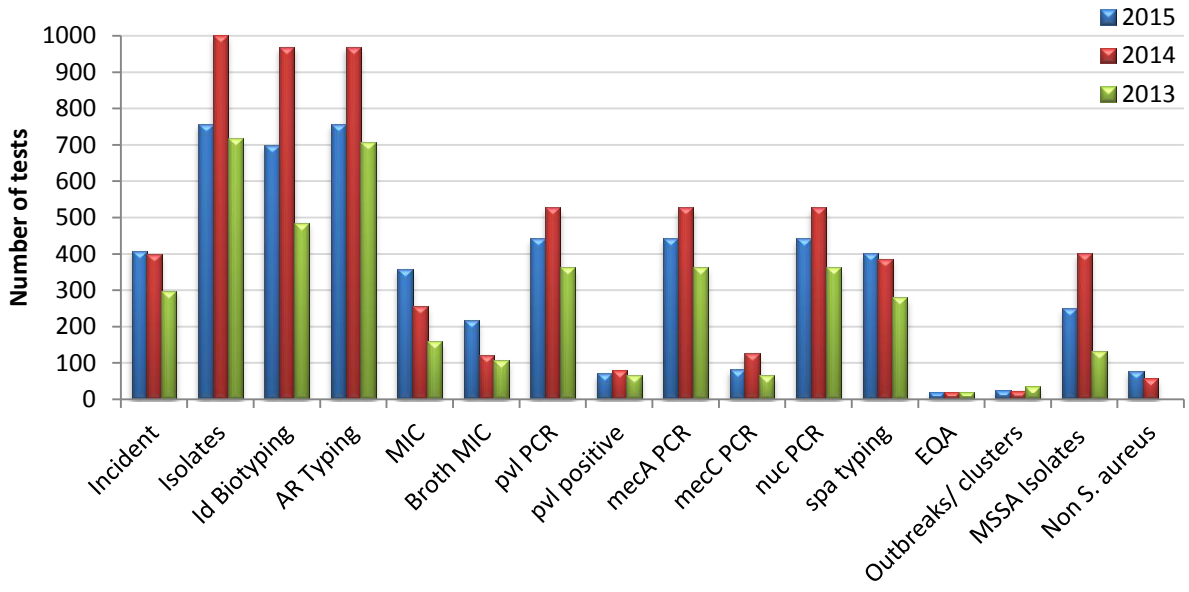
All isolates undergo Antibiogram-Resistogram (AR) typing performed in accordance with EUCAST methodology using a panel of 23 antimicrobial agents. Where interpretive criteria are not available from EUCAST, criteria set by the Clinical and Laboratory Standards Institute (CLSI) or developed in the NMRSARL 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 Number of isolates investigated in the National MRSA Reference Laboratory in recent years**




Isolates submitted for investigation as part of the European Antimicrobial Resistance Surveillance Network (EARS-Net) are the first *S. aureus* recovered from blood stream infections per patient per quarter and represent approximately 95% of the isolates submitted to the Health Protection Surveillance Centre. Reference Laboratory (Ref-Lab) isolates include those recovered during routine surveillance or skin and soft tissue infections. Significant increase evident in 2014 is due to assistance provided to 2 external hospitals monitoring all *S. aureus* isolates recovered within their institutions over a defined period of time.

**Figure 2 Investigation requests in the National MRSA Reference Laboratory in 2015 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. 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.

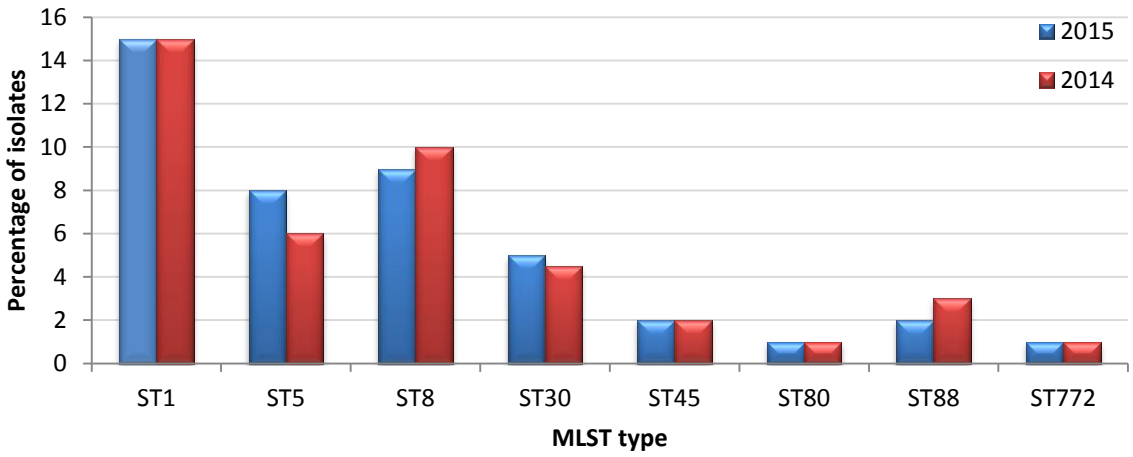
*spa* typing involves sequencing of the Staphylococcal protein A gene (*spa)* to recognise mutations or repeat insertion/deletion events that can cause changes in the polymorphic X region of the *spa* gene. It has become a well-established discriminatory method for outbreak investigations but has also 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 2015, 401 isolates were investigated. Isolates investigated by *spa* typing included 75 isolates recovered from blood culture specimens, 83 MSSA isolates and 89 PVL-positive isolates along with isolates involved in outbreaks and isolates exhibiting unusual phenotypic characteristics.

Among the isolates there were 123 different *spa* types recognised. As in previous years, there was great diversity seen among the MSSA isolates (50 types among 81 isolates) in comparison to the MRSA (83 *spa* types among 312 isolates).

Using the inferred MLST data available from the *spa* typing online database the most frequently recognised MLST types accounted for 42% of the isolates and, similar to 2015 include ST1, ST5, ST8, ST30, ST45, ST80, ST88 and ST772 (Figure 3). The most frequently occurring *spa* type was t127, associated with ST1 (14.7%, 46/312). While this strain was associated with a prolonged outbreak throughout 2014, increased awareness of the phenotypic characteristics of the strain in other hospitals has led to an increase in the number of isolates receipted in the NMRSARL. Typically this strain exhibits multi antibiotic resistance (aminoglycosides, mupirocin, tetracycline, fusidic acid, ciprofloxacin and/or erythromycin) however some isolates exhibit a susceptible profile with resistance only to ampicillin.

**Figure 3 Most frequently recognised MLST among MRSA isolates investigated by *spa* typing during 2015 in comparison to 2014**




As previously reported in Ireland, over time, a strain displacement has occurred resulting in the predominant ST22-MRSA-IV. This displacement has also been reported in other countries where, once community associated strains have now become the predominant hospital associated strains (USA 300 in America and ST772 in India). Many of the strains recognised in Ireland have been reported elsewhere and very often, these strains exhibit greater resistance and harbour more virulence genes than the ST22 strains and so close monitoring is required in order to control the spread of these strains in the hospital setting.

## 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 2015 the most frequently occurring *spa* type among the ST22 isolates, t032 continued to be the most predominant strain however other ST22 associated *spa* types included t022, t515, t223, t2945 and t020 were also recognised.

## 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 2015, 442 *S. aureus* isolates were tested for carriage of the *lukS-PV* and *lukF-PV* genes encoding for PVL and of these 16% were PVL-positive. These isolates were selected based on clinical presentation or phenotypic epidemiological typing data and included 212 meticillin-susceptible *S. aureus* and 230 MRSA isolates. While the percentage of MRSA isolates positive for PVL remained consistent with previous years (21.7%, 50/230), a slight increase was seen among MSSA isolates where it increased from seven percent of MSSA (17/220) were PVL-positive in 2014 compared to 11.8% (25/212) in 2015.

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, continues to predominant among PVL-positive MRSA isolates. Also associated with USA300, this *spa* type has been recognised among Irish PVL-positive isolates since 2007 and this strains continues to increase in predominance. 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

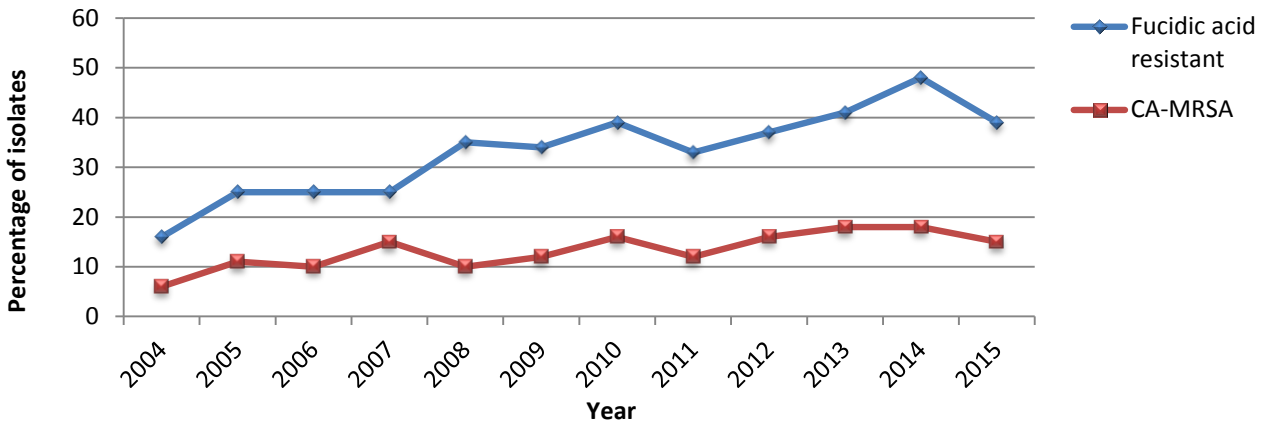
The phenotypic epidemiological typing techniques used in the NMRSARL enables the laboratory to monitor resistance among MRSA strains against clinically useful antimicrobial agents and to identify emerging resistance that may cause concern into the future with the EARS-Net isolates providing a representative 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 has continued in recent years however in 2015 a slight decrease was observed (Figure 4).

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. Of particular concern is the presence of *fusC* on mobile genetic elements such as SCC*mec* which has led to the suggestion that despite decreasing usage of the antibiotic in the UK this has resulted in increasing resistance rate (5).

**Figure 4 Percentage of MRSA isolates recovered from blood stream infections exhibiting resistance to fusidic acid**




The overall rate of resistance among MRSA isolates recovered from blood stream infections (blue) along with those which are considered to be CA-MRSA based on molecular typing of the isolate (red).

## 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 2015, 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 2015, mupirocin resistant was recognized among two percent (4/178) 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 2015 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 (6).

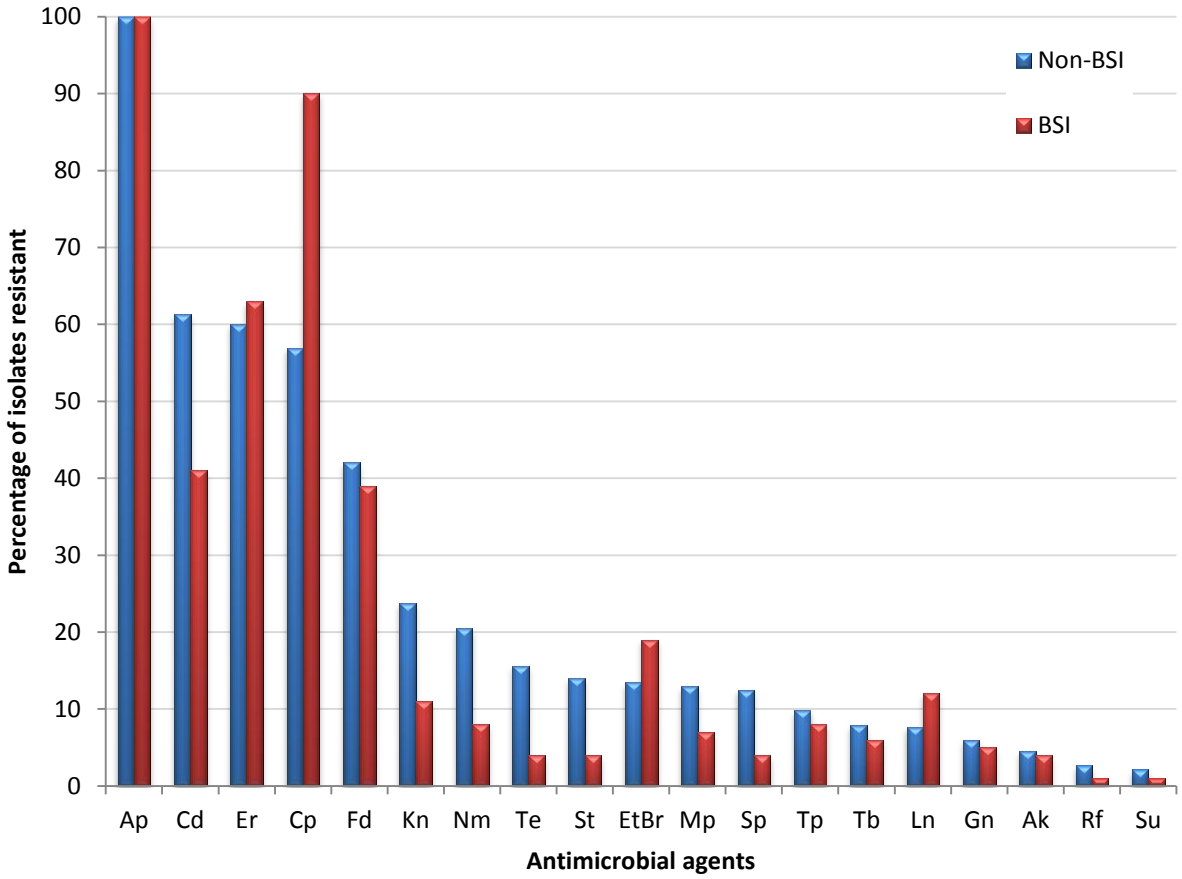
## Antimicrobial susceptibility among MRSA recovered from non- blood stream infections

While the previously mentioned rates of resistance relate only to EARS-Net isolates, a greater proportion of the work in the NMRSARL relates to isolates recovered from non-blood stream infections. In addition these isolates are often recovered from patients in the community where healthcare providers expect CA-MRSA.

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

Below shows the profile of all non-BSI isolates investigated in comparison to those of BSI isolates. Typically in Ireland ST22-MRSA-IV is the predominant HA-MRSA accounting for 80% of MRSA investigated under the EARS-Net project and exhibits resistance to ampicillin, ciprofloxacin, erythromycin and cadmium acetate. However the non-BSI isolates recovered both in healthcare facilities and in the community, and which may also be among others, ST22-MRSA-IV, exhibit higher levels of resistance against the panel of antibiotics tested with 82.34% of isolates exhibiting multi-antibiotic resistance, that is, resistance to three or more different classes of antibiotics and in particular to aminoglycosides, mupirocin and tetracycline.

**Figure 5 The percentage of blood stream isolates exhibiting resistance to each antimicrobial agent in comparison to those recovered from non-blood stream infections.**




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

## EDUCATION

The NMRSARL plays a prominent role in the education of laboratory staff and clinical staff.

In particular, NMRSARL staff gave lectures to undergraduate and post graduate students in the Dept. of Clinical Microbiology, TCD and the Dublin Institute of Technology. Scientific staff shared techniques used in the NMRSARL with staff from other hospital laboratories, research facilities, undergraduate students, transition year students and provided expert knowledge to students of other laboratories completing higher degrees.

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

## CONTINUOUS PROFESSIONAL DEVELOPMENT

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

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

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

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

One member of staff 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 or accepted for publication throughout the year.

# PUBLICATIONS

**Comparative Genotypes, Staphylococcal Cassette Chromosome *mec* (SCCmec) Genes and Antimicrobial Resistance amongst *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* Isolates from Infections in Humans and Companion Animals.**

McManus BA, Coleman DC, Deasy EC, Brennan GI, O' Connell B, Monecke S, Ehricht R, Leggett B, Leonard N, Shore AC. (2015)

PLoS One. 2015 Sep 17;10(9):e0138079. doi: 10.1371/journal.pone.0138079. eCollection 2015.

## Abstract

This study compares the characteristics of Staphylococcus epidermidis (SE) and Staphylococcus haemolyticus (SH) isolates from epidemiologically unrelated infections in humans (Hu) (28 SE-Hu; 8 SH-Hu) and companion animals (CpA) (12 SE-CpA; 13 SH-CpA). All isolates underwent antimicrobial susceptibility testing, multilocus sequence typing and DNA microarray profiling to detect antimicrobial resistance and SCCmec-associated genes. All methicillin-resistant (MR) isolates (33/40 SE, 20/21 SH) underwent dru and mecA allele typing. Isolates were predominantly assigned to sequence types (STs) within a single clonal complex (CC2, SE, 84.8%; CC1, SH, 95.2%). SCCmec IV predominated among MRSE with ST2-MRSE-IVc common to both Hu (40.9%) and CpA (54.5%). Identical mecA alleles and nontypeable dru types (dts) were identified in one ST2-MRSE-IVc Hu and CpA isolate, however, all mecA alleles and 2/4 dts detected among 18 ST2-MRSE-IVc isolates were closely related, sharing >96.5% DNA sequence homology. Although only one ST-SCCmec type combination (ST1 with a non-typeable [NT] SCCmec NT9 [class C mec and ccrB4]) was common to four MRSH-Hu and one MRSH-CpA, all MRSH isolates were closely related based on similar STs, SCCmec genes (V/VT or components thereof), mecA alleles and dts. Overall, 39.6% of MR isolates harbored NT SCCmec elements, and ACME was more common amongst MRSE and CpA isolates. Multidrug resistance (MDR) was detected among 96.7% of isolates but they differed in the prevalence of specific macrolide, aminoglycoside and trimethoprim resistance genes amongst SE and SH isolates. Ciprofloxacin, rifampicin, chloramphenicol [fexA, cat-pC221], tetracycline [tet(K)], aminoglycosides [aadD, aphA3] and fusidic acid [fusB] resistance was significantly more common amongst CpA isolates. SE and SH isolates causing infections in Hu and CpA hosts belong predominantly to STs within a single lineage, harboring similar but variable SCCmec genes, mecA alleles and dts. Host and staphylococcal species-specific characteristics were identified in relation to antimicrobial resistance genes and phenotypes, SCCmec and ACME.

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

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

Antimicrob Agents Chemother. 2016 Apr 22;60(5):3007-15. **(submitted 2015)**

## Abstract

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

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

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

PLoS One. 2016 Feb 17;11(2):e0149396. doi: 10.1371/journal.pone.0149396. (**submitted 2015)**

## Abstract

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

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

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

J Hosp Infect: 92 (2016) 287e292

## Abstract

Background

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

Aim

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

Methods

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

Findings

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

Conclusion

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

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

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

J Clin Microbiol. 2016 Feb;54(2):445-8. (accepted 2015)

## Abstract

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

**Characterization of methicillin-resistant *Staphylococcus aureus* from residents and the environment in a long-term care facility.**

Ludden C, Brennan G, Morris D, Austin B, O'Connell B and Cormican M.

Epidemiol Infect 02/2015; -1(14):1-4.

## Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health concern associated with residence in a long-term care facility (LTCF). The aim of this prospective study was to characterize MRSA isolated from residents over a 1-year period and their physical environment over a 2-year period. MRSA was recovered from 17/64 residents (R) of a LTCF and from 42 environmental (E) sites. All isolates carried the mecA gene and lacked the mecC and Panton-Valentine leukocidin (PVL) genes. Thirteen spa types were identified with t032 being the most frequent (41% of total; n = 8R, 16E), followed by t727 (22% of total; n = 13E), and t8783 (10% of total; n = 6E). Five spa types were each represented by single isolates. Thirty-nine isolates were of spa types associated with the multilocus sequence type ST22 (t032, 41%; spa-CC22, 68%) and reflect the predominance of ST22 in Irish hospitals. The uncommon spa types t727, t8783, t1372, t3130, t10038 were present in the environment but not detected in residents and are infrequently observed in Ireland.

# QUALITY MANAGEMENT SYSTEM

In 2015, 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 2015, >95% 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 2015 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 2015, 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.

Increased use of the DNA microarray in outbreak investigations was used to further differentiate ST22-MRSA-IV and other CA-MRSA strains recovered in healthcare facilities.

# RESOURCES

## Staff

During 2015 the staff working in the NMRSARL were;

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

Dr. Anna Shore continued in her role as a Lecturer in Applied and Translational Microbiology and, in this role continued her involvement in the development of applied research in MRSA between the School of Dental Science 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 €252,868. 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.

## 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

# BIBLIOGRAPHY

1. Shore AC, Rossney AS, Kinnevey PM Brennan OM, Creamer E, *et al.,* Enhanced discrimination of highly clonal ST22-methicillin-resistant Staphylococcus aureus IV isolates achieved by combining spa, dru, and pulsed-field gel electrophoresis typing data J Clin Microbiol. 2010 May;48(5):1839-52.
2. Frénay HM, Bunschoten AE, Schouls LM, van Leeuwen WJ, Vandenbroucke-Grauls CM, Verhoef J, Mooi FR. Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. Eur J Clin Microbiol Infect Dis. 1996 Jan;15(1):60-4.
3. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, *et al*. A Field Guide to Pandemic, Epidemic and Sporadic Clones of Methicillin-Resistant *Staphylococcus aureus*. PLoS ONE 2011; 6(4): e17936
4. Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC. 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. J Clin Microbiol. 2014 Mar;52(3):859-70
5. Ellington MJ, Reuter S, Harris SR, Holden MTG, Cartwright EJ, Greaves D, *et al*. Emergent and evolving antimicrobial resistance cassettes in community-associated fusidic acid and meticillin-resistant Staphylococcus aureus. Int J Antimicrob Agents 2015;45(5):477–84.
6. Shore AC, Brennan OM, Ehricht R, Monecke S, Schwarz S, Slickers P, *et al*. Identification and characterization of the multidrug resistance gene *cfr* in a Panton-Valentine leukocidin-positive sequence type 8 methicillin-resistant *Staphylococcus aureus* IVa (USA300) isolate. Antimicrob agents chemother. 2010;54(12):4978-84.