Emergence of Hospital- and Community-Associated Panton-Valentine Leukocidin-Positive Methicillin-Resistant *Staphylococcus aureus* Genotype

ST772-MRSA-V in Ireland and Detailed Investigation of a ST772-MRSA-V Cluster in a Neonatal Intensive Care Unit

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Sequence type (ST) 22 methicillin-resistant Staphylococcus aureus (MRSA) harboring staphylococcal cassette chromosome mec (SCCmec) IV (ST22-MRSA-IV) have predominated in Irish hospitals since the late 1990s. Six distinct clones of community-associated (CA-) MRSA have also been identified in Ireland. A new strain of CA-MRSA, ST772-MRSA-V, has recently emerged and become widespread in India and has spread into hospitals. In the present study, highly similar MRSA isolates were recovered from seven colonized neonates in a neonatal intensive care unit (NICU) in a maternity hospital in Ireland during 2010 and 2011, two colonized NICU staff, one of their colonized children and a NICU environmental site. The isolates exhibited multi-antibiotic resistance, spa type t657 and were assigned to ST772-MRSA-V by DNA microarray profiling. All isolates encoded resistance to macrolides (msrA and mpbBM) 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 following 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- and CA-ST772-MRSA-V is currently emerging in Ireland and may have been imported from India on several occasions.
Methicillin-resistant Staphylococcus aureus (MRSA) are among the most common causes of hospital-acquired infection worldwide, with elderly patients at increased risk of infection. Neonates in intensive care units are also at increased risk of MRSA infection or colonization. Risk factors for this group are premature birth, low birth weight, chronic underlying disease, prolonged exposure to antibiotics and invasive or surgical procedures (35). Since the 1990s the emergence and increasing prevalence of community-associated MRSA (CA-MRSA) outside of the healthcare environment has highlighted the changing epidemiology of MRSA (24). CA-MRSA have been associated with colonization of healthy individuals but can also cause skin and soft tissue infections and life-threatening necrotizing pneumonia in children and adults with no predisposing risk factors (13, 24). Outbreaks of CA-MRSA have been reported in specific community settings and groups including among those in prisons, crèches, gymnasia and military bases and among Australian Aboriginals and Native Americans (7, 13).

Methicillin-resistant S. aureus have acquired a mobile genetic element carrying the methicillin resistance genes mecA termed the staphylococcal cassette chromosome mec (SCCmec). Eleven different SCCmec elements have been described to date (19, 21, 39). Recently a new type of MRSA of animal origin harboring a novel and highly divergent mecA gene has been identified in Ireland, the UK and Germany (10, 18, 39).

Community-associated MRSA are generally genetically distinct to hospital-acquired (HA)-MRSA and are characterized by the presence of small SCCmec elements, usually SCCmec type IV, and to a lesser extent, SCCmec type V. Community-associated-MRSA are often less resistant to antibiotics than HA-MRSA and often express specific toxins and
virulence factors such as Panton-Valentine leukocidin (PVL) and phenol-soluble modulins (41). PVL is a bi-component pore-forming cytolytic toxin encoded by the \textit{lukF-PV} and \textit{lukS-PV} genes that are encoded by a group of specific bacteriophages (46).

The emergence of CA-MRSA clones was originally thought to be continent-specific but intercontinental spread of several CA-MRSA clones has been observed and new clones have also emerged (23). Recently, a PVL-positive CA-MRSA clone, ST772-MRSA-V, that is relatively multi-antibiotic resistant compared to other CA-MRSA clones, was identified in several countries. Methicillin-susceptible ST772 \textit{S. aureus} was originally reported in Bangladesh, but was quickly followed by reports of ST772-MRSA-V in India and Malaysia and subsequently in England, Italy, Australia, Germany, Hong Kong and Abu Dhabi (1, 11, 15, 24, 26, 34). Many patients identified with ST772-MRSA-V outside of India had familial links in, or travel history to or from, India, resulting in the ST772-MRSA-V clone being dubbed the Bengal Bay clone (15, 24, 26). In addition, ST772-MRSA-V is increasingly prevalent in India, where it has spread into hospitals and, along with ST22-MRSA-IV, has displaced the previously predominant nosocomial ST239-MRSA-III clone (11). There are also a number of other reports where CA-MRSA have also been associated with hospital-acquired infections and outbreaks among adults and neonates (12, 27, 28, 34, 36, 41, 45). It has been speculated that CA-MRSA infection among neonates is acquired through contact with colonized adults (36).

The spread of these CA-MRSA in both community and nosocomial settings have led to revised infection prevention and control guidelines to include environments such as gymasia, childcare facilities and prisons (29). The constant influx of patients, visitors and health-care workers, who constitute a reservoir for CA-MRSA, into hospitals place additional burdens on staff attempting to control the spread of HA-MRSA (28). Infection control interventions that
are successful in the control of HA-MRSA may be useful in the control of CA-MRSA outbreaks in hospitals, however these may need to be modified to include more emphasis on the involvement of healthcare workers in outbreaks (12, 22, 27).

MRSA has been endemic in Irish hospitals for almost four decades with a major shift in the predominant clonal type occurring in each decade (37). Since 2002, isolates belonging to ST22-MRSA-IV have predominated as the cause of nosocomial infections (33, 37, 38). Several genotypes of PVL-positive CA-MRSA have also been reported in Ireland and have been linked to the importation of different strains, with ST30-MRSA-IV and ST8-MRSA-IV predominating (33).

Here we report the recent emergence of ST722-MRSA-V in Irish hospitals and in the community. The study describes the detailed characterization by DNA microarray profiling and spa typing of closely related isolates of PVL-positive MRSA ST772-MRSA-V recovered in 2010 and 2011 from colonized neonates and staff in a neonatal intensive care unit in an Irish maternity hospital, and additional epidemiologically unrelated isolates of this strain from cases of HA- and CA- infection in Ireland between 2009-2011.
MATERIALS AND METHODS

Hospital Setting. The neonatal intensive care unit (NICU) described in this report is located in a 194-bed maternity hospital in Dublin, Ireland. The neonatal unit is a thirty-six bedded unit consisting of three air-conditioned wards and a separate isolation wing. Babies admitted to the unit include those born prematurely (before 37 weeks), those with congenital abnormalities and any baby who has problems identified immediately after birth or who subsequently becomes ill.

MRSA surveillance and description of the cluster. In October 2010, a skin swab from the chin of a baby in the NICU yielded MRSA. Subsequent screening swabs from the nose and umbilicus yielded MRSA, which were characterized as spa type t657 and ST772-MRSA-V. Within two weeks, two additional babies also yielded ST772-MRSA-V. Isolates were recovered from a nasal screening swab of the first baby and nasal and umbilicus screening swabs from the second. In an attempt to identify the source of the MRSA, nasal screening swabs were collected from NICU staff, who were closely involved in the care of the neonates, along with the parents of each baby positive for MRSA. At this time a source was not identified and there were no further ST772-MRSA-V isolates recovered from patients in the NICU until March 2011 when nose and umbilicus screening swabs from two babies yielded additional ST772-MRSA-V isolates. In May, a further two babies yielded ST772-MRSA-V, one from an umbilicus swab and from nasal and umbilicus swabs.

Following the isolation of the ST772-MRSA-V strain in March 2011, extensive supervised staff screening was undertaken. Nose, throat, groin and/or axilla screening specimens were collected from 148 hospital staff including medical, nursing and midwifery staff, maternity care assistants, household cleaning staff, administrative staff, radiology staff,
social workers and biomedical engineers. In addition, parents of MRSA-positive babies were screened and environmental specimens were also collected from horizontal surfaces within the unit around the cots of MRSA-positive babies and from staff areas.

**Confirmation of isolates as MRSA.** All MRSA isolates recovered from patients in the NICU during the seven-month period were submitted to the Irish National MRSA Reference Laboratory (NMRSARL) for epidemiological typing. On receipt of isolates in NMRSARL all MRSA isolates were inoculated onto Protect beads (Technical Service Consultants Limited, Heywood, United Kingdom) and stored at -70°C prior to subsequent investigation. Isolates were confirmed as *S. aureus* using the tube coagulase test and methicillin resistance was detected using 10 µg and 30-µg cefoxitin disks (Oxoid Ltd., Basingstoke, United Kingdom).

**Antibiogram-resistogram (AR) typing.** All isolates underwent AR typing using the Clinical and Laboratory Standards Institute (CLSI) standardised disk diffusion methodology as described previously (33). Antibiogram-resistogram typing involved determining the resistance of isolates to a panel of 23 antimicrobial agents including amikacin, ampicillin, cadmium acetate, chloramphenicol, ciprofloxacin, erythromycin, ethidium bromide, fusidic acid, gentamicin, kanamycin, lincomycin, mercuric chloride, mupirocin, neomycin, phenyl mercuric acetate, rifampicin, spectinomycin, streptomycin, sulphonamide, tetracycline, tobramycin, trimethoprim and vancomycin (30).

**Biotyping.** All MRSA isolates were characterized by a biotyping method that investigated hydrolysis of urea, hydrolysis of Tween 80, and pigment production, all as described previously (32).

**DNA macrorestriction digestion analysis.** DNA macrorestriction digestion analysis followed by pulsed-field gel electrophoresis (PFGE) was performed on all MRSA isolates as
described previously (33). Banding patterns were analysed using the GelCompar software package (version 4.1; Applied Maths, Belgium) (32) and the final interpretation of the differences between PFGE patterns was performed as recommended by Tenover et al. (44).

**Staphylococcal protein A (spa) typing.** spa typing, which involves PCR amplification and sequencing of the variable X region in the *S. aureus* protein A gene (*spa*), was performed as previously described (17) on MRSA isolates associated with the cluster and which exhibited an indistinguishable banding pattern when investigated by PFGE. The Ridom StaphType software (Ridom GmbH, Würzburg, Germany) was used for *spa* sequence analysis to identify the repeat successions of the *spa* gene and to assign it a *spa* type.

**DNA microarray analysis.** All MRSA isolates associated with the cluster were investigated by DNA microarray analysis using the StaphType Kit (Alere Technologies GmbH, Jena, Germany) to detect 334 *S. aureus* gene sequences and alleles including antimicrobial-resistance genes, virulence-associated genes and typing markers (23). The array also assigns isolates to a multilocus sequence type (ST) and/or clonal complex (CC) and SCCmec type (25).

**Multilocus sequence typing (MLST).** Three isolates underwent MLST to confirm the ST of the isolates, and was performed as described previously (16, 40). This included two isolates representative of the predominant *spa* type identified among the isolates (M11/0092 and M11/0085) as well as the one isolate exhibiting a different but closely related *spa* type (M10/0333).

**Prevalence of ST772-MRSA-V in Irish hospitals.** The database of isolates submitted to the NMRSARL was examined for other isolates that exhibited an AR pattern or *spa* type indistinguishable to the ST772-MRSA-V isolate from the NICU cluster. Seven additional
isolates were identified and were investigated by spa typing and DNA microarray analysis and, where possible, clinical information regarding diagnosis, travel history and ethnicity was collected retrospectively.
RESULTS

Cluster investigation. Over the seven-month period of the investigation (October 2010 to May 2011) seven babies in the NICU were found to be colonized by ST772-MRSA-V, i.e. none had clinical or other evidence to suggest MRSA infection. All parents and staff members screened for MRSA after the first isolates were recovered in October 2010 were found to be negative for MRSA. During the second episode of staff screening in March 2011, 148 staff were screened and five were identified as MRSA carriers, two of whom were found to carry an indistinguishable strain to ST772-MRSA-V recovered from the neonates. Of the two staff members, one was from India (yielded isolate M11/0092) and one from Ireland (yielded isolate M11/0097) and both had worked in the NICU at the time when the babies yielded positive MRSA cultures.

Epidemiological investigation indicated that the staff member of Indian background (yielded isolate M11/0092) was the probable index case due to her intimate involvement in the care for all babies who were colonized with ST772-MRSA-V. The staff member had worked in the NICU for a number of years. However she had been hospitalized during a visit to India where she had returned to give birth shortly prior to the recovery of the first ST772-MRSA-V isolate. Prior to decolonization treatment ST772-MRSA-V was recovered from her nose, groin and axilla but she had no symptoms of infection. Following decolonization treatment, undertaken by the occupational health department, screening swabs were negative for MRSA on two occasions. However, the ST772-MRSA-V strain was recovered from a groin swab from a third set of screening swabs. Family contacts of the probable index case were screened and a nasal swab from her Indian-born child yielded M11/0167. Table 1 shows the epidemiological characteristics of all ST772-MRSA-V isolates recovered during the NICU investigation.
The decolonisation protocol applied to staff members consisted of a five-day course of 2% mupirocin nasal ointment applied to the inner surface of each nostril three times daily and use of an octenidine-based shower gel/shampoo. Babies over 36-week gestation were also bathed in octenidine and treated with mupirocin. One of the staff members (yielded isolate M11/0097) colonized with the ST772-MRSA-V strain was successfully decolonized. The probable index case proved difficult to decolonize and as such underwent the decolonization protocol twice. Following decolonization a screening throat swab collected from her child yielded the same ST772-MRSA-V strain. Treatment for this throat carriage was not possible using a mouth wash due to the age of the child.

**Environmental screening.** Following environmental screening of approximately 30 areas including the main reception, staff kitchen and clinical areas, MRSA was recovered from one area of the NICU, a horizontal surface in the area of one baby associated with the ST772-MRSA-V cluster. The environmental cleaning regimen, which included the cleaning of all equipment in the patient zone area, (e.g. monitors, suction machines, intravenous pumps, canopy of the incubator etc.) with detergent wipes and which was carried out daily within the unit, was increased to twice daily.

**Characterization of MRSA isolates.** The 11 MRSA isolates from patients, staff members, the child of the probable index case and the environmental isolate from the NICU exhibited similar multi-antibiotic resistance phenotype (Table 1) and were positive for tween 80 and urease hydrolysis. The PFGE banding patterns exhibited by these 11 isolates were indistinguishable. All MRSA isolates exhibited *spa* type t657 and DNA microarray analysis assigned the isolates to CC1 and ST573/772 with SCC*meC* type V. MLST of three isolates investigated confirmed the genotype as ST772. All isolates harbored multiple antibiotic
resistance genes including those encoding resistance to macrolides ($msrA$ and $mpbBM$) and aminoglycosides ($aacA$-$aphD$ and $aphA3$) (Table 1). All isolates were positive for the PVL genes $lukF$-PV and $lukS$-PV, the enterotoxin genes sea, sec and sel, the enterotoxin gene cluster ($egc$) along with the immune evasion complex (IEC) gene $scn$ but the β-toxin gene $hlb$ was not detected, either in its intact or disrupted form (Table 1). Genes encoding adhesion and biofilm factors were also detected (Table 1).

**Prevalence of ST772-MRSA-V in Irish hospitals.** Comparison of the AR pattern of the ST772-MRSA-V strain recovered in the NICU to all MRSA isolates submitted to the NMRSARL since 2000 showed that a similar AR pattern had previously been identified among seven additional sporadic PVL-positive isolates, two recovered from patients in two separate Irish hospitals and five from community sources (Table 1). These isolates were confirmed as ST772-MRSA-V by DNA microarray analysis. Six isolates were assigned $spa$ type t657 while one was assigned $spa$ type t345 (Table 1). MLST of the latter isolate confirmed it as ST772. The DNA microarray data for these isolates was indistinguishable to that of the ST772-MRSA-V isolates recovered in the NICU (Table 1).

Three of the seven patients from whom the isolates were recovered had Indian parents while one other patient had traveled to India as part of his work (Table 1). The latter patient had a history of recurrent boils and at the time of sampling had boils on his elbows, buttocks, thighs and face. Five of the seven ST772-MRSA-V isolates were recovered from samples submitted by general medical practitioners in the community, however, two patients acquired the strain while in hospital. There was no known epidemiological association between any of the seven patients from whom the additional ST772-MRSA-V were recovered.
The present study describes the detection and detailed molecular characterization of a cluster of the PVL-positive and multi-antibiotic resistant CA-MRSA strain ST772-MRSA-V among staff and patients in an Irish neonatal intensive care unit and the subsequent retrospective identification of isolates of this strain from patients in other hospitals and in the community in Ireland. Epidemiological data identified that the likely source of the ST772-MRSA-V cluster in the NICU as a staff member from India. The use of spa typing in conjunction with the DNA microarray was essential in directing the epidemiological investigation, identifying the likely source and hence, the implementation of control measures that led to prevention of further spread of the ST772-MRSA-V strain. Antibiogram-resistogram typing indicated that there was a possible outbreak or a cluster of MRSA within the NICU and this was confirmed by PFGE and DNA microarray analysis. However the ST772-MRSA-V isolates recovered from the NICU were not associated with infections. All ST772-MRSA-V isolates identified in this study exhibited the same multi-antibiotic resistant AR pattern that was distinctly different to that exhibited by the most frequently occurring MRSA clone in Irish hospitals (ST22-MRSA-IV) (3). Using spa typing all of the isolates apart from one were assigned to spa type t657. The remaining isolate was assigned to spa type t345, which differs in one repeat unit only to spa type t657. DNA microarray analysis and MLST assigned the isolates as ST772-MRSA-V and identified that they harbored similar virulence and antimicrobial resistance genes to each other and to previously reported ST772-MRSA-V isolates. However, unlike some of the recently reported ST772-MRSA-V isolates the isolates in the present study lacked the antimicrobial resistance genes \textit{erm(C)} and \textit{tet(K)} (24) and the enterotoxin genes \textit{sek} and \textit{seq} (34). It is interesting to note the absence of DNA microarray
signals corresponding to the presence of either the disrupted or complete beta hemolysin gene \textit{hlb} in the ST772-MRSA-V isolates in this and previous studies (24, 34). Most human \textit{S. aureus} isolates including MRSA harbor a disrupted \textit{hlb} gene due to insertional inactivation during lysogenization by \textit{hlb}-converting bacteriophages (8, 9). These findings indicate the possible presence of mutations in the primer or probe binding sites within \textit{hlb} used with the DNA microarray system in isolates of this strain. The presence of the IEC gene \textit{scn} that is encoded on bacteriophages that integrate within \textit{hlb} suggests that \textit{hlb} is present in a truncated form in these isolates. Additional studies are underway to investigate this further.

Unlike other ST772-MRSA-V isolates reported in the literature (1, 11, 12, 15, 24, 26, 34) those recovered within the Irish maternity hospital NICU were not associated with any known clinical diagnosis of infection and were most frequently recovered from screening specimens of patients within the NICU. In contrast the seven ST772-MRSA-V isolates recovered from patients in other Irish hospitals (n=2) and from patients in the community (n=5) and for which there was clinical information available were found to be associated with skin and soft tissue infections.

The increased spread of CA-MRSA in India has been associated with severe soft tissue infections however there has been an increase in the number of cases associated with bacteraemias affecting neonates (11). The decreasing prevalence of the HA-MRSA strain ST239-MRSA-III in hospitals in India since 2006 coupled with an increase in prevalence of ST22-MRSA-IV and ST772-MRSA-V has led to the suggestion that these strains may be replacing the ST239-MRSA strain in Indian hospitals (11). Within Ireland it has been shown that previously predominant strains of MRSA in Irish hospitals have also been replaced at different time periods by different strains. In 1989 the most frequently occurring strain was
ST239-MRSA-III while in 1993 this had changed to ST8-MRSA-II (31, 37). The most recent change in strains occurred in 1998 where the frequency of ST22-MRSA-IV had increased and this strain now accounts for 80% of MRSA bloodstream infections investigated in the NMRSARL under the European Antimicrobial Resistance Surveillance Network (EARS-Net, previously EARSS) in 2003 (32). Although the predominant MRSA strain causing bloodstream infections has not changed since 1998 the proportion of \( S. \) aureus isolates that are resistant to methicillin has fallen from 41.9% in 2006 to 24.3% in 2010 (4). The ST22-MRSA-IV clone could be displaced at some stage in the future, possibly by ST772-MRSA-V, which, as the situation in India has shown, is capable of displacing previously successful nosocomial MRSA strains.

Transmission of MRSA between healthcare workers and patients has been reported on many occasions (2, 5, 6, 20, 42, 48). Current Irish guidelines recommend that staff are screened for MRSA carriage only as part of an outbreak investigation (43). However, it has been suggested that there are higher colonization rates in settings where MRSA is endemic and randomized periodic screening may be required to identify asymptomatic persons carrying MRSA (7, 14). Similar to the practice in the Netherlands of screening all patients who have previously been hospitalized in foreign countries, identifying and screening high-risk staff with previous hospitalization or a MRSA-positive family contact should be considered (47).

The toddler associated with the index case in the present study yielded MRSA from a throat swab and as it is not possible to treat a toddler for carriage of MRSA in the throat, this may very likely lead to recolonization of the healthcare worker thus posing many challenges about further management.
The emergence of a PVL-positive and multi-antibiotic resistant MRSA strain in the community and hospitals in Ireland is a worrying development and enhanced surveillance is vital to ensure that these strains do not spread. The familial links or travel history of a number of patients from which the ST772-MRSA-V strain was recovered from in the present study suggests that the isolates recovered in Ireland may have been imported from India and that this may have occurred on a number of different occasions. Foreign travel as well as the employment of healthcare staff from foreign countries may lead to further importation incidents of this or other MRSA strains into Ireland. Rapid and informative molecular typing, such as provided by DNA microarray profiling, is essential for the early identification of MRSA strains and to prevent these strains spreading in hospitals.
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and characterization of an ST97-SCCmec-V community-associated meticillin-resistant
*Staphylococcus aureus* clone in a neonatal intensive care unit and special care baby


### TABLE 1: Epidemiological, clinical, phenotypic and genotypic characteristics of the hospital-associated and community-associated ST772-MRSA-V isolates recovered in Ireland between 2009 and 2011

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Isolate no.</th>
<th>MRSA-positive site</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Relevant clinical Information</th>
<th>Location acquired/source</th>
<th>spa type</th>
<th>Antimicrobial resistance pattern&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antimicrobial resistance genes&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Virulence associated genes&lt;sup&gt;b&lt;/sup&gt;</th>
<th>agr/ capsule type&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>M10/0338</td>
<td>Nasal, umbilicus, perineum</td>
<td>Nasal</td>
<td>7 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>In patient</td>
<td>t657</td>
<td>AMP&lt;sup&gt;d&lt;/sup&gt;, CIP&lt;sup&gt;e&lt;/sup&gt;, GEN, KAN, TOH, TMP</td>
<td>blaZ, mph(A), aacA&lt;sub&gt;-&lt;/sub&gt; aphD, aphA&lt;sub&gt;3&lt;/sub&gt; &amp; sat, fosB, sdrM</td>
<td>lukF-PV &amp; lukS-PC, sea, sec &amp; sel, ege (vag, sei, sem, sen, seo &amp; sea), scc, bhp, clfA, clfB, cna, efbh, ebpS, eno, fih, fibA, fibB, map, tcaA, icad, icac, sdrC, sdrD, vwb, susG</td>
</tr>
<tr>
<td>H1</td>
<td>M10/0342</td>
<td>Nasal, umbilicus</td>
<td>Nasal</td>
<td>15 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>In patient</td>
<td>t657</td>
<td>AMP&lt;sup&gt;d&lt;/sup&gt;, CIP&lt;sup&gt;d&lt;/sup&gt;, GEN, KAN, TOH, TMP</td>
<td>blaZ, mph(A), aacA&lt;sub&gt;-&lt;/sub&gt; aphD, aphA&lt;sub&gt;3&lt;/sub&gt; &amp; sat, fosB, sdrM</td>
<td>lukF-PV &amp; lukS-PC, sea, sec &amp; sel, ege (vag, sei, sem, sen, seo &amp; sea), scc, bhp, clfA, clfB, cna, efbh, ebpS, eno, fih, fibA, fibB, map, tcaA, icad, icac, sdrC, sdrD, vwb, susG</td>
</tr>
<tr>
<td>H1</td>
<td>M10/0349</td>
<td>Nasal, umbilicus</td>
<td>Nasal</td>
<td>20 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>In patient</td>
<td>t657</td>
<td>AMP&lt;sup&gt;d&lt;/sup&gt;, CIP&lt;sup&gt;e&lt;/sup&gt;, GEN, KAN, TOH, TMP</td>
<td>blaZ, mph(A), aacA&lt;sub&gt;-&lt;/sub&gt; aphD, aphA&lt;sub&gt;3&lt;/sub&gt; &amp; sat, fosB, sdrM</td>
<td>lukF-PV &amp; lukS-PC, sea, sec &amp; sel, ege (vag, sei, sem, sen, seo &amp; sea), scc, bhp, clfA, clfB, cna, efbh, ebpS, eno, fih, fibA, fibB, map, tcaA, icad, icac, sdrC, sdrD, vwb, susG</td>
</tr>
<tr>
<td>H1</td>
<td>M11/0082</td>
<td>Nasal, umbilicus</td>
<td>Nasal</td>
<td>3 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>In patient</td>
<td>t657</td>
<td>AMP&lt;sup&gt;d&lt;/sup&gt;, CIP&lt;sup&gt;e&lt;/sup&gt;, GEN, KAN, TOH, TMP</td>
<td>blaZ, mph(A), aacA&lt;sub&gt;-&lt;/sub&gt; aphD, aphA&lt;sub&gt;3&lt;/sub&gt; &amp; sat, fosB, sdrM</td>
<td>lukF-PV &amp; lukS-PC, sea, sec &amp; sel, ege (vag, sei, sem, sen, seo &amp; sea), scc, bhp, clfA, clfB, cna, efbh, ebpS, eno, fih, fibA, fibB, map, tcaA, icad, icac, sdrC, sdrD, vwb, susG</td>
</tr>
<tr>
<td>H1</td>
<td>M11/0085</td>
<td>Nasal, umbilicus</td>
<td>Nasal</td>
<td>24 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>In patient</td>
<td>t657</td>
<td>AMP&lt;sup&gt;d&lt;/sup&gt;, CIP&lt;sup&gt;e&lt;/sup&gt;, GEN, KAN, TOH, TMP</td>
<td>blaZ, mph(A), aacA&lt;sub&gt;-&lt;/sub&gt; aphD, aphA&lt;sub&gt;3&lt;/sub&gt; &amp; sat, fosB, sdrM</td>
<td>lukF-PV &amp; lukS-PC, sea, sec &amp; sel, ege (vag, sei, sem, sen, seo &amp; sea), scc, bhp, clfA, clfB, cna, efbh, ebpS, eno, fih, fibA, fibB, map, tcaA, icad, icac, sdrC, sdrD, vwb, susG</td>
</tr>
<tr>
<td>H1</td>
<td>M11/0107</td>
<td>Umbilicus</td>
<td>Nasal</td>
<td>9 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>In patient</td>
<td>t657</td>
<td>AMP&lt;sup&gt;d&lt;/sup&gt;, CIP&lt;sup&gt;e&lt;/sup&gt;, GEN, KAN, TOH, TMP</td>
<td>blaZ, mph(A), aacA&lt;sub&gt;-&lt;/sub&gt; aphD, aphA&lt;sub&gt;3&lt;/sub&gt; &amp; sat, fosB, sdrM</td>
<td>lukF-PV &amp; lukS-PC, sea, sec &amp; sel, ege (vag, sei, sem, sen, seo &amp; sea), scc, bhp, clfA, clfB, cna, efbh, ebpS, eno, fih, fibA, fibB, map, tcaA, icad, icac, sdrC, sdrD, vwb, susG</td>
</tr>
<tr>
<td>H1</td>
<td>M11/0120</td>
<td>Nasal, axilla, groin</td>
<td>Nasal</td>
<td>9 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>In patient</td>
<td>t657</td>
<td>AMP&lt;sup&gt;d&lt;/sup&gt;, CIP&lt;sup&gt;e&lt;/sup&gt;, GEN, KAN, TOH, TMP</td>
<td>blaZ, mph(A), aacA&lt;sub&gt;-&lt;/sub&gt; aphD, aphA&lt;sub&gt;3&lt;/sub&gt; &amp; sat, fosB, sdrM</td>
<td>lukF-PV &amp; lukS-PC, sea, sec &amp; sel, ege (vag, sei, sem, sen, seo &amp; sea), scc, bhp, clfA, clfB, cna, efbh, ebpS, eno, fih, fibA, fibB, map, tcaA, icad, icac, sdrC, sdrD, vwb, susG</td>
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<tr>
<td>H1</td>
<td>M11/0092</td>
<td>Nasal, axilla, groin</td>
<td>Nasal</td>
<td>29 years</td>
<td>Indian</td>
<td>Staff member, probable index case</td>
<td>Staff working in NICU</td>
<td>t657</td>
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<td></td>
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<tr>
<td>H1</td>
<td>M11/0097</td>
<td>Unknown</td>
<td>Nasal</td>
<td>35 years</td>
<td>Irish</td>
<td>Staff working in NICU</td>
<td>Staff working in NICU</td>
<td>t657</td>
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<tr>
<td>H1</td>
<td>M11/0167</td>
<td>Nasal</td>
<td>Nasal</td>
<td>16 months</td>
<td>Indian</td>
<td>Child of the probable index case</td>
<td>Family contact of colonized NICU staff member</td>
<td>t657</td>
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<tr>
<td>Hospital</td>
<td>Isolate no.</td>
<td>MRSA-positive site</td>
<td>Age</td>
<td>Ethnicity</td>
<td>Relevant clinical Information</td>
<td>Location acquired/source</td>
<td>Antimicrobial resistance pattern&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Antimicrobial resistance genes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Virulence associated genes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>agr/ capsule type&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>H1</td>
<td>M11/0093</td>
<td>Environment</td>
<td>N/A</td>
<td>N/A</td>
<td>Horizontal surface in NICU&lt;sup&gt;c&lt;/sup&gt;</td>
<td>N/A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>t657</td>
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<tr>
<td></td>
<td>M11/0035</td>
<td>Unknown</td>
<td>9 years</td>
<td>Indian</td>
<td>Pustule on back in patient with no previously positive MRSA screen</td>
<td>Community-associated</td>
<td>t657</td>
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<tr>
<td>H2</td>
<td>M09/0243</td>
<td>Unknown</td>
<td>82 years</td>
<td>Unknown</td>
<td>In patient with no previously positive MRSA screen</td>
<td>Community-associated</td>
<td>t657</td>
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<tr>
<td></td>
<td>M10/0045</td>
<td>Unknown</td>
<td>29 years</td>
<td>Unknown</td>
<td>Community-associated</td>
<td>t657</td>
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<td></td>
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<tr>
<td>H3</td>
<td>M10/0203</td>
<td>Unknown</td>
<td>96 years</td>
<td>Indian</td>
<td>Eye swab</td>
<td>In patient</td>
<td>Community-associated</td>
<td>t657</td>
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<td></td>
<td>M10/0131</td>
<td>Unknown</td>
<td>28 years</td>
<td>Indian</td>
<td>Unknown</td>
<td>Community-associated</td>
<td>t657</td>
<td>t657</td>
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<tr>
<td></td>
<td>M10/0361</td>
<td>Unknown</td>
<td>18 months</td>
<td>Indian</td>
<td>Ear swab</td>
<td>Community-associated</td>
<td>t657</td>
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<tr>
<td></td>
<td>M10/0033</td>
<td>Unknown</td>
<td>22 years</td>
<td>Irish with history of travel to India</td>
<td>History of recurrent boils and abscesses</td>
<td>Community-associated</td>
<td>t345</td>
<td></td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 23 antimicrobial agents including AMI, amikacin; AMP, ampicillin; CAD, cadmium acetate; chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; ethidium bromide; fusidic acid; GEN, gentamicin; KAN, kanamycin; lincomycin; mercuric chloride; mupirocin; NEO, neomycin; phenyl mercuric acetate; rifampin; spectinomycin; streptomycin; sulfonamide; tetracycline; TOB, tobramycin; TMP, trimethoprim; vancomycin.

<sup>b</sup> Antimicrobial resistance genes, virulence-associated genes, agr type and capsule type determined using the StaphyType DNA microarray kit (Alere, Germany).

<sup>c</sup> NICU, neonatal intensive care unit.

<sup>d</sup> 14/18 isolates exhibited moderate resistance to amikacin while four isolates (M11/0035, M10/0045, M10/0131 and M10/0203) were susceptible.
4/18 isolates exhibited resistance to cadmium acetate (M10/0342, M10/0349, M10/0131 and M11/0107), two (M11/0035 and M10/0033) were susceptible and the remaining 12 isolates exhibited moderate resistance.

17/18 isolates exhibited resistance to ciprofloxacin and one isolate (M11/0035) was susceptible.

All isolates exhibited moderate resistance to erythromycin.

N/A, not applicable.