Emergence of Hospital- and Community-Associated Panton-Valentine Leukocidin-Positive Methicillin-Resistant *Staphylococcus aureus* Genotype ST772-MRSA-V in Ireland and Detailed Investigation of an ST772-MRSA-V Cluster in a Neonatal Intensive Care Unit

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

Methicillin-resistant *Staphylococcus aureus* (MRSA) is 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 the health care environment have highlighted the changing epidemiology of MRSA (24). CA-MRSA has 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 (12, 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 Aborigines and Native Americans (7, 12).

Methicillin-resistant *S. aureus* has acquired a mobile genetic element carrying the methicillin resistance genes *mecA* that has been 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 was identified in Ireland, the United Kingdom, and Germany (10, 18, 39).

Community-associated MRSA is generally genetically distinct from hospital-acquired MRSA (HA-MRSA) and is characterized by the presence of small SCCmec elements, usually SCCmec type IV, and to a lesser extent SCCmec type V. Community-associated MRSA is often less resistant to antibiotics than HA-MRSA and often expresses specific toxins and virulence factors, such as Panton-Valentine leukocidin (PVL) and phenol-soluble modulins (41). PVL is a bicomponent pore-forming cytolytic toxin encoded by the *lukF-PV* and *lukS-PV* genes, which are carried 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 multiantibiotic resistant compared to other CA-MRSA clones was identified in several countries. Methicillin-susceptible ST772 *S. aureus* was originally reported in Bangladesh, but this 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, 13, 15, 19, 20, 21, 22, 23, 24).
Those born prematurely (before 37 weeks), those with congenital abnormalities, those with maternal infections, and those who have spent time in the hospital for any reason (24, 26, 34). Many patients identified with ST772-MRSA-V outside 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 (13). There have also been a number of other reports where CA-MRSA was associated with hospital-acquired infections and outbreaks among adults and neonates (11, 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 isolates in both community and nosocomial settings has led to revised infection prevention and control guidelines to include environments such as gymnasium, child care facilities, and prisons (29). The constant influx of patients, visitors, and health care workers, who constitute a reservoir for CA-MRSA, into hospitals places 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 health care workers in outbreaks (11, 22, 27).

MRSA has been endemic in Irish hospitals for almost 4 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 ST772-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 infections in Ireland between 2009 and 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 36-bed 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 organisms that were characterized as spa type t657 and ST772-MRSA-V. Within 2 weeks, two additional babies also yielded ST772-MRSA-V. Isolates were recovered from a nasal screening swab of the first additional baby and from 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 that 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 one 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 7-month period were submitted to the Irish National MRSA Reference Laboratory (NMRSARL) for epidemiological typing. On receipt of isolates at NMRSARL, all MRSA isolates were inoculated onto Protec beads (Technical Service Consultants Ltd., Heywood, United Kingdom) and stored at ~70°C prior to subsequent investigation. Isolates were confirmed as S. aureus by using the tube coagulase test, and methicillin resistance was detected using 10-μg and 30-μg cefoxitin disks (Oxoid Ltd., Basingstoke, United Kingdom).

AR typing. All isolates underwent antibiogram-resistogram (AR) typing using the Clinical and Laboratory Standards Institute (CLSI) standardized disk diffusion methodology as described previously (33). Antiidiogram-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, rifampin, spectinomycin, streptomycin, sulfonamide, 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 analyzed 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 that 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 assigned isolates to a multilocus sequence type (ST) and/or clonal complex (CC) and SCCmec type (25).

MLST. Three isolates underwent multilocus sequence typing (MLST) to confirm the ST of the isolate, and MLST 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 from the ST772-MRSA-V isolate from the NICU cluster. Seven additional isolates were identified and 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 7-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 5 were identified as MRSA carriers, 2 of whom were found to carry a strain indistinguishable from ST772-MRSA-V recovered from the neonates. Of the two staff members, one was from India (and yielded isolate M11/0092) and one was from Ireland (and yielded isolate M11/0097); 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 (who 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 India-born child yielded M11/0167. Table 1 shows the epidemiological characteristics of all ST772-MRSA-V isolates recovered during the NICU investigation.

The decolonization protocol applied to staff members consisted of a 5-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 weeks' gestation were also bathed in octenidine and treated with mupirocin. One of the staff members (who 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 by use of 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 1 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 a similar multiantibiotic resistance phenotype (Table 1) and were positive for Tweak 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 SCCmec 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 [msr(A) and mprB(BM)] 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, and the enterotoxin gene cluster (egc) along with the immune evasion complex (IEC) gene scp, but the beta-hemolysin 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 were indistinguishable from those 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 isolates were recovered.

DISCUSSION

The present study describes the detection and detailed molecular characterization of a cluster of the PVL-positive and multiantibiotic-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 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 recov-
<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</th>
<th>Antimicrobial resistance genes</th>
<th>Virulence-associated genes</th>
<th>age/capsule type</th>
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</thead>
<tbody>
<tr>
<td>H1</td>
<td>M10/0338</td>
<td>Nasal, umbilicus, perineum</td>
<td>7 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>Inpatient</td>
<td>M10/0338</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>H1/5</td>
</tr>
<tr>
<td>H1</td>
<td>M10/0342</td>
<td>Nasal</td>
<td>15 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>Inpatient</td>
<td>M10/0342</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
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<td>H1/5</td>
</tr>
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<td>M10/0349</td>
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<td>20 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>Inpatient</td>
<td>M10/0349</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>H1/5</td>
</tr>
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<td>M11/0082</td>
<td>Nasal, umbilicus</td>
<td>3 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>Inpatient</td>
<td>M11/0082</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>H1/5</td>
</tr>
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<td>M11/0085</td>
<td>Nasal, umbilicus</td>
<td>24 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>Inpatient</td>
<td>M11/0085</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>H1/5</td>
</tr>
<tr>
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<td>M11/0107</td>
<td>Umbilicus</td>
<td>9 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>Inpatient</td>
<td>M11/0107</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>H1/5</td>
</tr>
<tr>
<td>H1</td>
<td>M11/0120</td>
<td>Nasal, umbilicus</td>
<td>9 days</td>
<td>Irish</td>
<td>Carriage cluster in NICU</td>
<td>Inpatient</td>
<td>M11/0120</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
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<td>H1</td>
<td>M11/0092</td>
<td>Nasal, axilla, groin</td>
<td>29 yrs</td>
<td>Indian</td>
<td>Staff member, probable index case</td>
<td>Staff working in NICU</td>
<td>M11/0092</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
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<td>M11/0097</td>
<td>Unknown</td>
<td>35 yrs</td>
<td>Irish</td>
<td>Staff member</td>
<td>Staff working in NICU</td>
<td>M11/0097</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>H1/5</td>
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<tr>
<td>H1</td>
<td>M11/0167</td>
<td>Nasal</td>
<td>16 mos</td>
<td>Indian</td>
<td>Child of probable index case</td>
<td>Staff working in NICU</td>
<td>M11/0167</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
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<td>H1/5</td>
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<td>H1</td>
<td>M11/0093</td>
<td>Environment</td>
<td>NA</td>
<td>NA</td>
<td>Horizontal surface in NICU</td>
<td>NA</td>
<td>M11/0093</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
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<tr>
<td>NA*</td>
<td>M11/0035</td>
<td>Unknown</td>
<td>9 yrs</td>
<td>Indian</td>
<td>Pustule on back</td>
<td>Community associated</td>
<td>M11/0035</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
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<tr>
<td>H2*</td>
<td>M09/0243</td>
<td>Unknown</td>
<td>82 yrs</td>
<td>Unknown</td>
<td>In patient with no previously positive MRSA screen</td>
<td>Patient</td>
<td>M09/0243</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
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<td>NA*</td>
<td>M10/0045</td>
<td>Unknown</td>
<td>29 yrs</td>
<td>Unknown</td>
<td>Eye swab</td>
<td>Community associated</td>
<td>M10/0045</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
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<td>H3*</td>
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<td>96 yrs</td>
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<td>Ear swab</td>
<td>Community associated</td>
<td>M10/0203</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
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<tr>
<td>NA*</td>
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<td>28 yrs</td>
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<td>M10/0131</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>Community associated</td>
</tr>
<tr>
<td>NA*</td>
<td>M10/0361</td>
<td>Unknown</td>
<td>18 mos</td>
<td>Indian</td>
<td>Ear swab</td>
<td>Community associated</td>
<td>M10/0361</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>Community associated</td>
</tr>
<tr>
<td>NA*</td>
<td>M10/0033</td>
<td>Unknown</td>
<td>22 yrs</td>
<td>Irish with history of travel to India</td>
<td>History of recurrent boils and abscesses</td>
<td>M10/0033</td>
<td>AMI, AMP, CAD, CIP, ERY, GEN, KAN, NEO, TOB, TMP</td>
<td>blaZ, msr(A), mph(C), aacA-aphD, aphA3 and sat, fosB, sdrM</td>
<td>lukF-PV and lukS-PV, sea, sec, and sel, egc</td>
<td>Community associated</td>
<td></td>
</tr>
</tbody>
</table>

*Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 23 antimicrobial agents including amikacin (AMI), ampicillin (AMP), cadmium acetate (CAD), chloramphenicol, ciprofloxacin (CIP), erythromycin (ERY), ethidium bromide, fusidic acid, gentamicin (GEN), kanamycin (KAN), lincomycin, mercuric chloride, muiracacin, neomycin (NEO), phenyl mercuric acetate, rifampicin, spectinomycin, streptomycin, sulfamamide, tetracycline, tobramycin (TOB), trimethoprim (TMP), and vancomycin.

†Antimicrobial resistance genes, virulence-associated genes, agr type, and capsule type were determined using the StaphyType DNA microarray kit (Alere, Germany).
‡Fourteen of 18 isolates exhibited moderate resistance to amikacin, while 4 isolates (M11/0035, M10/0045, M10/0131, and M10/0203) were susceptible.
§Four of 18 isolates exhibited resistance to cadmium acetate (M10/0342, M10/0349, M10/0131, and M11/0107), 2 (M11/0035 and M10/0033) were susceptible, and the remaining 12 isolates exhibited moderate resistance.
∥Seventeen of 18 isolates exhibited resistance to ciprofloxacin, and 1 isolate (M11/0035) was susceptible.
*Eleven isolates exhibited moderate resistance to erythromycin.
NA, not applicable.
EGC, evasion gene cluster consisting of seg, sei, sem, sen, seo, and seu.
ered from the NICU were not associated with infections. All ST772-MRSA-V isolates identified in this study exhibited the same multiantibiotic-resistant AR pattern, which was distinctly different from that exhibited by the most frequently occurring MRSA clone in Irish hospitals (ST22-MRSA-IV) (3). Based on spa typing, all of the isolates apart from one were assigned to spa type t657. The remaining isolate was assigned to spa type t145, which differs in one repeat unit only from spa type t657. DNA microarray analysis and MLST assigned the isolates as ST772-MRSA-V and showed 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 *erm(C)* and *tet(K)* (24) and the enterotoxin genes *sek* and *seq* (34). The absence of DNA microarray signals corresponding to the presence of either the disrupted *hlb* and the enterotoxin genes *sek* and *seq* (34). The absence of DNA microarray signals corresponding to the presence of either the disrupted or complete beta-hemolysin gene *hlb* in the ST772-MRSA-V isolates in this and previous studies (24, 34) is interesting. Most human *S. aureus* isolates, including MRSA, harbor a disrupted *hlb* gene due to insertional inactivation during lysogenization by *hlb*-converting bacteriophages (8, 9). These findings indicate the possible presence of mutations in the primer or probe binding sites within *hlb* used with the DNA microarray system in isolates of this strain. The presence of the IEC gene *scr*, which is carried on bacteriophages that integrate within *hlb*, suggests that *hlb* is present in a truncated form in these isolates. Additional studies are under way to investigate this further.

Unlike other ST772-MRSA-V isolates reported in the literature (1, 11, 13, 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 bacteremias affecting neonates (13). 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 (13). 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, when 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 EARS) 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 fell 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 health care workers and patients has been reported on many occasions (2, 5, 6, 20, 42, 48). Current Irish guidelines recommend that staff be 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 very likely led to recolonization of the health care worker, thus posing many challenges regarding further management.

The emergence of a PVL-positive and multiantibiotic-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 histories of a number of patients from which the ST772-MRSA-V strain was recovered in the present study suggest 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 health care 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 that provided by DNA microarray profiling, is essential for the early identification of MRSA strains and for prevention of these strains spreading in hospitals.

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REFERENCES


