

## SHORT REPORT

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

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### SUMMARY

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 Pantone–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.

**Key words:** Environment, long-term care facility (LTCF), methicillin-resistant *S. aureus* (MRSA), molecular epidemiology, *spa* typing.

*Staphylococcus aureus* is a ubiquitous microorganism which has been isolated from humans, animals, and the environment [1, 2]. While *S. aureus* can colonize the skin and nasal passages of 20–30% of people without apparent adverse impact on health [3], it can also cause a wide variety of infections including pneumonia, bloodstream infection, skin and soft tissue infection, and food poisoning [3, 4] and, in some

circumstances, can produce toxins increasing the severity of these infections.

Although the proportion of *S. aureus* bloodstream infections attributed to methicillin-resistant *S. aureus* (MRSA) has declined in recent years it remains a challenge with particular clonal lineages of health-care associated (HA)-MRSA, notably 'epidemic' [EMRSA-15 (ST22) and EMRSA-16 (ST36)], and the community-associated (CA-MRSA) strains such as USA300 (ST8) [2, 3, 5]. In Ireland, multilocus sequence type (MLST) (ST22) accounts for 70–80% of blood stream MRSA infections [5]. Residence in a long-term care facility (LTCF) is a recognized risk factor for acquisition of MRSA [1, 6, 7]. While MRSA

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can be transmitted from person-to-person via direct skin contact, environmental surfaces contaminated with MRSA also play a role in transmission owing to the ability of some strains to survive on inanimate surfaces for several months [6]. Following on from an earlier study of 64 residents of a LTCF where nasal colonization with MRSA was detected in 17 residents (C. Ludden, unpublished data), we set out to assess the relationship between MRSA from the residents and their corresponding environment.

In September 2011, the residents were moved to a newly built LTCF. Multiple swabs of the environment (door handles, tables, floor surfaces, armchairs, bed-frames, bed-side lockers, on-call buttons, handles beside showers, toilet flushers, toilet seats, tap handles and railings beside common toilets) were collected prior to decommissioning of the original LTCF (August 2011) and both before and after occupation by residents (August–November 2011 and August 2013) in the new facility.

Environmental samples were collected using Copan ESswabs (BS ISO 18 593:2004) which were inoculated into peptone water and incubated overnight. Ten microlitres of peptone water was plated onto chromID™ MRSA agar (bioMérieux, France). Nasal specimens were placed in transport medium and inoculated directly onto chromID™ MRSA agar. All chromID™ MRSA agar plates were incubated at 37 °C and examined for MRSA growth after 24 h and 48 h. Suspect isolates were confirmed by latex agglutination (Pastorex Staph Plus, Bio-Rad, France). Isolates were confirmed as methicillin resistant using ceftioxin and EUCAST methodology and interpretive criteria.

Isolates were stored on Protect beads (Technical Service Consultants Ltd, UK) at –70 °C prior to subsequent detailed analysis. All isolates were tested for susceptibility to 19 antimicrobials by disc testing including amikacin (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), fusidic acid (10 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (2 µg), mupirocin (5 µg), neomycin (30 µg), rifampicin (5 µg), spectinomycin (500 µg), streptomycin (25 µg), sulphonamide (300 µg), tetracycline (30 µg), tobramycin (10 µg), trimethoprim (5 µg) and vancomycin (30 µg) [4].

Isolates were screened for *mecA*, *mecC* and genes encoding Pantone–Valentine leukocidin (PVL) using real-time polymerase chain reaction and *spa* gene typing as previously described [8]. *spa* sequences were analysed using Ridom StaphType software (Ridom GmbH, Germany) and types were clustered into *spa* clonal complexes using based upon repeat pattern (BURP) analysis

where *spa* types were excluded if they contained <5 repeat successions and if the cost (number of genetic events) was >5. Where possible an MLST type was inferred from data available in an online database (Ridom *spa* server <http://www.spaserver.ridom.de/>).

MRSA was recovered from 82/201 (40%) and from 12/69 (17%) environmental samples collected in 2011 and 2013, respectively; all sites were positive for MRSA except the tap handles, railings beside common toilets, and on-call buttons. The lower frequency of MRSA detection in 2013 might have been due to better infrastructure in the new LTCF. The first MRSA recovered from nasal swabs from each resident ( $n = 17$ ) together with 42 environmental isolates were selected for epidemiological typing. Environmental isolates were chosen to reflect the diversity of MRSA isolated over time and different locations within each LTCF and included one isolate from each positive site, with the exception of one that was no longer viable; 19 and 12 isolates from 2011 and 2013, respectively, were recovered from the newly built LTCF along with 11 isolates recovered from the old facility.

All isolates carried the *mecA* gene and lacked the *mecC* and PVL genes *lukF-PV* and *lukS-PV*. The resistance profile of isolates is shown in Table 1. In total, 13 *spa* types were recognized: t032 (24/59), t727 (13/59), t8783 (6/59), t022 (3/59), t002 (2/59), t020 (2/59), t1372 (2/59), and t379 (2/59). There was a single isolate each of *spa* types t611, t4623, t045, t3130, and t10038. BURP analysis clustered 40 isolates from 14 residents and 26 environmental specimens in a single *spa* clonal complex (*spa*-CC22) (Table 1) and this complex represented environmental isolates collected in 2011 and 2013. Types t002, t045, t3130 were excluded from clustering as the cost was greater than >5, and t727, t1372 were excluded from clustering as they consisted of four and two repeat succession units, respectively. These five *spa* types were defined as singletons.

To our knowledge this is the first study to report the typing of isolates recovered from all colonized residents in a single facility along with isolates recovered from their associated environment over an extended period. A single *spa* type, t032, accounted for 41% of isolates. *spa* types associated with ST22, the ST which overall accounted for 66% (39/59) of all isolates totalled 82% (14/17) from residents and 60% (25/42) from the environment. In Ireland ST22 has been the most common lineage in hospitals since 2002 and accounts for 70–80% of MRSA isolates from bloodstream infections [5], therefore this predominance was not unexpected. *spa* type t727 is associated with ST45 (Ridom *spa* server

Table 1. *spa* types, repeat succession, *spa* inferred MLST, clonal complexes, PCR results and antimicrobial susceptibility profiles for 59 environment and clinical MRSA

<i>Spa</i> type	<i>N</i> (% total MRSA)	Clinical (% total)	Environment (% total)	<i>spa</i> repeat succession	<i>spa</i> clonal complex	Inferred MLST	PVL	<i>mecA</i>	Antibiogram*
t032	24 (41%)	8 (47%)	16 (38%)	26-23-23-13-23-31-29-17-31-29-17-25-17-25-16-28	22	ST22	Neg.	Pos.	AMP,CIP, FUS (16), ERY (16), TET (1)
t022,	3 (5%)	3 (17%)	0 (0%)	26-23-13-23-31-29-17-31-29-17-25-17-25-16-28	22	ST22	Neg.	Pos.	AMP, CIP, ERY
t020	2 (3%)	0 (0%)	2 (5%)	26-23-31-29-17-31-29-17-25-17-25-16-28	22	ST22	Neg.	Pos.	AMP, CIP, ERY, FUS
t379	2 (3%)	1 (6%)	1 (2%)	26-23-23-13-23-31-29-17-25-17-25-16-28	22	ST22	Neg.	Pos.	AMP, CIP, ERY, FUS
t611	1 (2%)	1 (6%)	0 (0%)	26-23-23-13-23-31-17-31-29-17-25-17-25-16-28	22	ST22	Neg.	Pos.	AMP, CIP, FUS
t4623	1 (2%)	1 (6%)	0 (0%)	26-23-13-23-31-29-132-17-31-29-17-25-17-25-16-28	22	ST22	Neg.	Pos.	AMP
t8783	6 (10%)	0 (0%)	6 (15%)	26-23-13-23-31-29-17-31-29-17-31-17-25-16-28	22	ST22	Neg.	Pos.	AMP, CIP, ERY (2)
t10038	1 (2%)	0 (0%)	1 (2%)	26-23-23-13-23-31-29-17-31-340-17-25-17-25-16-28	22	None	Neg	Pos.	AMP,CIP, FUS
t002	2 (3%)	2 (12%)	0 (0%)	26-23-17-34-17-20-17-12-17-16	Singleton	ST5, ST231	Neg.	Pos.	AMP, CIP, ERY,
t045	1 (2%)	1 (6%)	0 (0%)	26-17-20-17-12-17-16	Singleton	ST5, ST225	Neg.	Pos.	AMI, AMP, CIP, ERY, KAN, LIN, NEO, SPC, TOB, FUS
t3130	1 (2%)	0 (0%)	1 (2%)	26-23-23-13-16-16-28	Singleton	None	Neg.	Pos.	AMP, CIP, ERY, FUS
t727	13 (22%)	0 (0%)	13 (31%)	08-16-02-43	Singleton	ST45	Neg.	Pos.	AMP, CIP, ERY, FUS (10)
t1372	2 (3%)	0 (0%)	2 (5%)	26-28	Singleton	None	Neg.	Pos.	AMP, CIP, FUS

MLST, Multilocus sequence typing; PCR, polymerase chain reaction; PVL, Pantón–Valentine leucocidin; Neg., negative; Pos., positive.

\* Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 23 antimicrobial agents including amikacin (AMI), ampicillin (AMP), chloramphenicol, ciprofloxacin (CIP), erythromycin (ERY), fusidic acid (FUS), gentamicin, kanamycin (KAN), lincomycin (LIN), mupirocin, neomycin (NEO), rifampin, spectinomycin (SPC), streptomycin, sulfonamide, tetracycline (TET), tobramycin (TOB), trimethoprim and vancomycin.

<http://www.spaserver.ridom.de/>) but no ST could be inferred for types t3130 and t1372 (Table 1).

ST5 [t002 ( $n = 2$ ) and t045 ( $n = 1$ )] exhibiting multi-antimicrobial resistance was identified in MRSA from three residents but was not found in the environment (Table 1). *spa* type t002 (ST5) has previously been isolated from residents of LTCFs in California, where it accounted for 23% of isolates, although t008 type (ST8) predominated in the corresponding hospitals [7]. A study in Israel identified ST5 as the predominant strain in residents of LTCFs and staff which was also common in Israeli hospitals [9]. Unfortunately, in the current study, the environment of the rooms of residents carrying MRSA ST5 were not sampled and so we are unable to speculate further on the origin of this clone. The finding of MRSA in a newly built facility is interesting. As MRSA was not detected until commissioning of the old building commenced it is possible that healthcare workers and/or transfer of items may have contributed to the introduction of MRSA into the environment. As healthcare workers were not screened for MRSA colonization their contribution to environmental contamination in both facilities remains unknown.

*spa* type t727 (ST45) was recovered from the environment of the old facility (13/42) in August 2011 and in August 2013 from the new building but it was not detected in residents. Based on the *spa* types submitted to the Ridom *spa* Server (<http://spaserver.ridom.de/>, 2 December 2014, date last accessed) *spa* type t727 has only been previously reported from Norway ( $n = 13$ ) and Ireland. During the 1-year study, MRSA isolates from two of four new residents colonized when first tested were of two unique *spa* types (t045 and t4623) and were not found in any long-term resident. These strains may represent new variants introduced from outside the LTCF into the setting of well-established resident strains.

The antimicrobial resistance profiles of the ST22 isolates reported here correlate with previous reports for this clone with observed resistances ranging from one to five antimicrobials. All but one of the 39 isolates of this ST were ciprofloxacin resistant which is consistent with HA-MRSA in contrast to CA-MRSA [2, 4].

In summary the single clonal complex/sequence type (*spa*-CC22) that predominates in both residents and the environment corresponds to that which causes most bloodstream infections in hospitals in Ireland. Two findings merit follow-up. Two of four colonized new residents carry minority MRSA variants and it would be of interest to determine if such residents acquire resident LTCF strains over time. Moreover, *spa* type t727 was

found in the environment over a 2-year period and t8783 was found throughout the old building in 2011, but was not identified in any resident. Further studies investigating the role of LTCF admissions from hospitals and the impact of environmental contamination are required to gain a broader understanding into the epidemiology of MRSA in such settings.

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## DECLARATION OF INTEREST

None

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