SHORT REPORT
Emergence of MRSA clone ST22 in healthy young adults in the community in the absence of risk factors

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SUMMARY
One thousand adults aged between 18 and 35 years were investigated for nasal colonization with community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA). Each volunteer completed a questionnaire to assess the presence or absence of risk factors for hospital-acquired MRSA (HA-MRSA) carriage. All MRSA isolated were characterized by microbiological and molecular methods. A S. aureus carriage rate of 22% and a MRSA carriage rate of 0.7% were observed. Analysis of the questionnaires revealed 121 individuals with HA-MRSA risk factors. Subsequently two MRSA infections with associated risk factors were excluded from calculation of the true carriage rate and an adjusted rate of 0.57% (5/879) was established. All seven MRSA isolates expressed the genotypic profile ST22-MRSA-IV, were PVL negative, agr type 1, and differed only by their antimicrobial susceptibility patterns. ST22-MRSA-IV (EMRSA-15) has shown worldwide spread in the hospital setting but has not been previously documented in isolation in the community.

Key words: Clinical microbiology, community outbreaks, hospital-acquired (nosocomial) infections, methicillin-resistant S. aureus (MRSA).

Staphylococcus aureus, the most virulent Staphylococcus species, is also the most prevalent pathogen isolated from hospitalized patients and the second most common from patients in outpatient settings [1]. Methicillin-resistant S. aureus (MRSA) first emerged in the 1960s and since then has been commonly seen in hospitals (HA-MRSA), clinics, and, more recently, in the community (CA-MRSA) [2]. CA-MRSA infections can be caused by new strains of MRSA which are genetically and phenotypically distinct from the typical multidrug-resistant (MDR) HA-MRSA. The CDC distinguishes CA-MRSA from HA-MRSA if

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the incidence of MRSA infections between 1997 and 2002, from 1–2% to 40% of all S. aureus infections. These two types of MRSA are particularly adapted to cause infections in hospitals compared to other MRSA strains, but as yet the basis for this is unknown [4].

The purpose of this study was to determine the prevalence of MRSA in the community in a population of young, asymptomatic adults in the absence of HA-MRSA risk factors and to subsequently characterize any isolates using both microbiological and molecular methods.

Between February and December 2008 the anterior nares of 1000 healthy university students (aged between 18 and 35 years) were sampled professionally (by a qualified nurse or by the study coordinator under nurse’s supervision) using Amies transport swabs (Sarstedt, UK). All participants were asked to complete an anonymous questionnaire which contained questions related to key demographics which would highlight any HA-MRSA risk factors, i.e. age, sex, residence, sporting activities, illicit drug use, medical history and contact with high-risk individuals. Ethical approval for the study was obtained from the Research Ethics Committee of Cork Institute of Technology.

Each participant had the distal part of the anterior nares sampled for culture and each swab was cultured for the isolation of S. aureus on mannitol salt agar (MSA; Cruinn Diagnostics, Ireland), and on MSA containing 4 μg/ml oxacillin (MSA-Ox) for MRSA. Suspected S. aureus colonies were identified using standard microbiological methods including Gram stain, coagulase and DNAse. All S. aureus isolates were maintained at room temperature on nutrient agar slopes.

DNA was extracted from all S. aureus isolates using the KeyPrep spin bacterial genomic DNA kit (Anachem Ltd, UK) according to the manufacturer’s instructions. PCR for the presence of the mecA and mec gene determinants was performed as previously described [5].

Antimicrobial susceptibility testing was performed on confirmed MRSA isolates against the following antimicrobials: cefoxitin (Cef), erythromycin (E), fusidic acid (Fd), tetracycline (Te), mupirocin (Mup), ciprofloxacin (Cip), teicoplanin (Tec), rifampicin (Rd), gentamicin (Cn) and linezolid (Liz), by disc diffusion method according to CLSI guidelines [6]. Resistance to oxacillin (Ox) was quantified by Etest (AB Biodisk, Sweden).

SCCmec element typing was performed using a multiplex PCR which tests eight loci as previously described [7]. Subtyping of type IV isolates was performed according to the method of Milheiro et al. [8]. Agr types and the presence of pvl genes were determined according to Lina et al. [9, 10]. MLST was performed using seven housekeeping genes as previously described [11]. An allelic profile (allele number) was obtained from the multilocus sequence typing website (http://www.mlst.net/).

Following analysis of 1000 nasal swabs, an S. aureus colonization rate of 22% was determined, 21.3% were methicillin-sensitive S. aureus (MSSA) and 0.7% MRSA. Using the questionnaires it was determined that 121 volunteers had HA-MRSA associated risk factors. Of the seven MRSA two had risk factors, in the first case (CIT-365), the student had undergone hand surgery which required a hospital stay in the previous 12 months and the second volunteer (CIT-524) was in regular contact with a known MRSA-positive patient. An adjusted CA-MRSA carriage rate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oxacillin (Etest μg/ml)</th>
<th>SCCmec</th>
<th>SCCmec IV subtype</th>
<th>PVL type</th>
<th>agr type</th>
<th>MLST (ST)</th>
<th>R type</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIT-365*</td>
<td>4</td>
<td>IV</td>
<td>n.t.</td>
<td>—</td>
<td>1</td>
<td>22</td>
<td>FdCip</td>
</tr>
<tr>
<td>CIT-373</td>
<td>4</td>
<td>IV</td>
<td>IVh</td>
<td>—</td>
<td>1</td>
<td>22</td>
<td>ECip</td>
</tr>
<tr>
<td>CIT-405</td>
<td>4</td>
<td>IV</td>
<td>IVh</td>
<td>—</td>
<td>1</td>
<td>22</td>
<td>FdCip</td>
</tr>
<tr>
<td>CIT-524*</td>
<td>16</td>
<td>IV</td>
<td>IVh</td>
<td>—</td>
<td>1</td>
<td>22</td>
<td>ECip</td>
</tr>
<tr>
<td>CIT-624</td>
<td>16</td>
<td>IV</td>
<td>IVh</td>
<td>—</td>
<td>1</td>
<td>22</td>
<td>Cip</td>
</tr>
<tr>
<td>CIT-676</td>
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<td>IV</td>
<td>IVh</td>
<td>—</td>
<td>1</td>
<td>22</td>
<td>ECip</td>
</tr>
<tr>
<td>CIT-771</td>
<td>96</td>
<td>IV</td>
<td>IVh</td>
<td>—</td>
<td>1</td>
<td>22</td>
<td>—†</td>
</tr>
</tbody>
</table>

MLST, Multi-locus sequence typing; Fd, fusidic acid; Cip, ciprofloxacin; E, erythromycin; n.t., not typed.
* Isolates that were excluded as CA-MRSA as these volunteers had HA-MRSA risk factors.
† Sensitive to all antibiotics in panel.
of 0.57% (5/879) was established following exclusion of these subjects. From the questionnaires it was observed that all five CA-MRSA carriers, who had no identifiable risk factors for HA-MRSA acquisition, were members of a sports team or a gymnasium.

The seven MRSA strains demonstrated oxacillin MIC values between 4 and 96 μg/ml, and showed four distinct antimicrobial susceptibility profiles, none of which was MDR. Molecular characterization identified all isolates to be ST22-MRSA-IV, PVL negative and agr type 1. Results are shown in Table 1.

ST22-MRSA-IV first emerged in the UK in 1991 and is still one of the most dominant epidemic clones in that country [1]. Since then its spread in the hospital setting has been reported in many countries, e.g. Italy, Portugal, Germany, Singapore, and it is currently established as the major clone in the Czech Republic and Majorca [1]. This study identified an asymptomatic carriage rate of 0.5% ST22-MRSA-IV in the community in young, healthy individuals with no identifiable hospital or healthcare risk association. In 2008, an Irish study showed that the epidemiological types that comprise the Irish hospital MRSA population changed between 1999 and 2003, whereby a non-MDR strain, ST22-MRSA-IV, displaced the previously predominant MDR genotype, ST8-MRSA-II [12]. Although the emergence and spread of ST22-MRSA-IV in a hospital setting has been widely documented, its prevalence in the community in a population of young, healthy individuals in the absence of HA-MRSA risk factors and any notable illness has not previously been reported to our knowledge. The asymptomatic carriage of ST22-MRSA-IV is interesting as it has been previously documented that persons who are carriers of MRSA and MSSA are at risk for subsequent infections with these organisms [13]. All five MRSA isolated in the absence of HA risk factors were from volunteers with an association with sport but analysis of the questionnaires revealed no link between them as none were from the same gymnasium or team. Isolation of MRSA in the community from individuals involved with sports with no HA-MRSA risk factors has been previously documented [13]. ST22-MRSA-IV is characteristically non-MDR and this lack of resistance has been attributed to its carriage of a smaller SCCmec element; SCCmec IV [14]. In recent years, MRSA strains have become associated with resistance to ciprofloxacin with a rate of ciprofloxacin resistance in MRSA in the UK averaging 94.6% and this can be as high as 98%.

However, studies have shown that ciprofloxacin-susceptible strains are increasing in prevalence and susceptibility in ST22-MRSA-IV strains has been documented [15]. These findings are supported by this study as one of the five CA-MRSA strains isolated was ciprofloxacin sensitive.

The identification of the asymptomatic nasal carriage of ST22-MRSA-IV in this study is worrying as it is clear that the distinction between CA-MRSA and HA-MRSA is fading as ST22-MRSA-IV originated in the hospital setting. This clone is well associated with nosocomial spread, and asymptomatic carriage by healthy subjects may in turn lead to a further increase in infection rates if these colonized individuals are admitted to hospital. It is clear from this study that CA-MRSA has become established in the wider community which underlines the need for continued surveillance to minimize the risk of future outbreaks.

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DECLARATION OF INTEREST

None.

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