

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

An observational cross-sectional study of nasal staphylococcal species of medical students of diverse geographical origin, prior to healthcare exposure. Prevalence of SCCmec, fusC, fusB and the arginine catabolite mobile element (ACME) in the absence of selective antibiotic pressure.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020391
Article Type:	Research
Date Submitted by the Author:	02-Nov-2017
Complete List of Authors:	Budri, Paulo; Royal College of Surgeons in Ireland, Clinical Microbiology Shore, Anna; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Coleman, David; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Kinnevey, Peter; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Humpreys, Hilary; Royal College of Surgeons in Ireland, Clinical Microbiology; Beaumont Hospital, Microbiology Department, Fitzgerald-Hughes, Deirdre; Royal College of Surgeons in Ireland, Clinical Microbiology
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Epidemiology
Keywords:	MRSA, antimicrobial resistance, nasal colonization, Coagulase negative staphylococci, <i>Staphylococcus aureus</i>

SCHOLARONE™
Manuscripts

1
2
3 1 **An observational cross-sectional study of nasal staphylococcal species of**
4
5 2 **medical students of diverse geographical origin, prior to healthcare exposure.**
6
7 3 **Prevalence of SCCmec, fusC, fusB and the arginine catabolite mobile element**
8
9 4 **(ACME) in the absence of selective antibiotic pressure.**
10

11 5
12
13
14 6 Paulo Eduardo Budri^{1*}, Anna C. Shore², David C. Coleman², Peter M. Kinnevey², Hilary
15
16 7 Humphreys^{1,3}, Deirdre Fitzgerald-Hughes¹.
17
18
19 8

20
21 9 ¹Department of Clinical Microbiology, Royal College of Surgeons in Ireland, Education
22
23 10 and Research Centre, Beaumont Hospital, Dublin 9, Ireland.

24
25 11 ²Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University
26
27 12 Hospital, University of Dublin, Trinity College, Dublin 2, Ireland

28
29
30 13 ³Microbiology Department, Beaumont Hospital, Dublin 9, Ireland
31
32
33 14

34
35 15 **Keywords;** *Staphylococcus aureus*, Coagulase-negative staphylococci, Nasal
36
37 16 colonization, Healthy human nares, Antimicrobial resistance, MRSA.

38
39
40 17 **Running title:** Antimicrobial resistance and virulence genes among staphylococcal
41
42 18 carriage isolates.
43
44
45 19

46
47 20 *Corresponding author: Paulo Eduardo Budri, Department of Clinical Microbiology,
48
49 21 RCSI Education and Research Centre, Smurfit Building, Beaumont Hospital, Dublin 9,
50
51 22 Ireland. Tel. +353 1 8093711, Fax +353 1 8092871, Email paulobudri@rcsi.com
52
53
54 23
55
56 24

1
2
3 25 **Abstract**
4

5 26 **Objective:** The aim of this study was to investigate co-located nasal *S. aureus* and
6
7 27 CoNS (mainly *S. epidermidis*) recovered from healthy medical students in their pre-
8
9 28 clinical year, prior to exposure to the healthcare environment, for the carriage of genes
10
11 29 and genetic elements common to both species and that may contribute to *S. aureus*
12
13 30 and MRSA evolution.

14
15 31 **Design:** Prospective observational cross-sectional study. Carriage of antimicrobial
16
17 32 resistance and virulence-associated genes in the absence of significant antibiotic
18
19 33 selective pressure was investigated among healthy medical students from
20
21 34 geographically diverse origins who were nasally co-colonised with *S. aureus* and
22
23 35 CoNS. Clonal lineages of *S. aureus* isolates were determined.

24
25 36 **Setting/Participants:** Dublin-based international undergraduate medical students

26
27 37 **Results:** Nasal *S. aureus* carriage was identified in 137/444 (30.8%) students of whom
28
29 38 nine (2%) carried MRSA (ST59-MRSA-IV (6/9), CC1-MRSA-V-SCC*fus* (3/9)). The
30
31 39 genes *mecA*, *fusB*, *ileS2*, *qacA/qacC* and ACME-*arc* were detected among colonizing
32
33 40 nasal staphylococci and had a significantly greater association with CoNS than *S.*
34
35 41 *aureus*. The rate of co-carriage of any of these genes in *S. aureus*/CoNS pairs
36
37 42 recovered from the same individual was <1 %.

38
39 43 **Conclusions** The relatively high prevalence of these genes among CoNS of the
40
41 44 healthy human flora in the absence of significant antibiotic selective pressure is of
42
43 45 interest. Further research is required to determine what factors are involved and
44
45 46 whether these are modifiable to help prevent the emergence and spread of antibiotic
46
47 47 resistance amongst staphylococci.
48

49 48

50 49

51 50

52 51
53
54
55
56
57
58
59
60

52 **Strengths and limitations of this study:**

- 53 • Evaluation of resistance gene carriage among Staphylococci in healthy medical
54 students in preclinical years.
- 55 • Evidence of antibiotic resistance among Staphylococci in the absence of
56 selective pressure.
- 57 • Study was carried out in a single centre.
- 58 • CoNS was investigated only in students co-colonised with *S. aureus*.
- 59 • The study design did not facilitate follow-up of this cohort during clinical training.

61 **Introduction**

62 *Staphylococcus aureus* and *Staphylococcus epidermidis* are significant colonisers of
63 healthy human skin and nares and are among the leading causes of healthcare-
64 associated infection (HAI). Morbidity, mortality and the financial burden associated with
65 methicillin-resistant *S. aureus* (MRSA) infections are well documented. Furthermore,
66 coagulase-negative staphylococci (CoNS) including *S. epidermidis* are reported
67 reservoirs of antimicrobial-resistance genes and their associated mobile genetic
68 elements, most notably the staphylococcal cassette chromosome (SCC) harbouring the
69 *mec* gene (SCC*mec*) [1].

70 Twelve SCC*mec* types and numerous subtypes have been described among
71 MRSA isolates to date. The more prevalent and diverse range of SCCs and SCC*mec*
72 among CoNS further supports CoNS as a reservoir for antimicrobial resistance genes
73 [1]. The identification of SCC, SCC*mec* and SCC-associated elements with other
74 antimicrobial and virulence genes and their epidemiological relationships among
75 clinical staphylococci has advanced our understanding of the role of CoNS in the
76 evolution of MRSA [2]. For example, the fusidic acid resistance gene *fusC* is
77 associated with SCC*mec*IV-SCC₄₇₆ and other SCC-like elements have been identified
78 in *S. aureus*, MRSA and CoNS and may contribute to MRSA emergence in countries

1
2
3 79 with significant fusidic acid usage. [3-5] Furthermore, the SCC-like arginine catabolic
4
5 80 mobile element (ACME) which enhances acid tolerance, is abundant among clinical
6
7 81 CoNS isolates, in particular *S. epidermidis* and *S. haemolyticus* [6]. Among *S. aureus*,
8
9 82 ACME has mainly been detected among isolates of the community-associated (CA)
10
11 83 USA300 clone [7]. CoNS are also a putative reservoir of the high level mupirocin
12
13 84 resistance encoding gene *ileS2*, which is also increasing among *S. aureus*/MRSA in
14
15 85 healthcare and community environments related to horizontal gene transfer or
16
17 86 expansion of specific clones [8 9].

18
19 87 Increasingly, MRSA clones previously associated with the community, such as CC1 are
20
21 88 spreading to healthcare settings making the differentiation between healthcare-
22
23 89 associated (HA) MRSA and CA-MRSA unclear [10]. Therefore, detailed investigation of
24
25 90 the genetic and phenotypic traits of colonizing staphylococcal species in community
26
27 91 settings are important to identify those with features that may contribute to their
28
29 92 evolution into potentially successful and formidable healthcare-associated clones. The
30
31 93 aim of this study was to investigate co-located nasal *S. aureus* and CoNS (mainly *S.*
32
33 94 *epidermidis*) recovered from healthy medical students in their pre-clinical year, prior to
34
35 95 exposure to the healthcare environment, for the carriage of genes and genetic
36
37 96 elements common to both species and that may contribute to *S. aureus* and MRSA
38
39 97 evolution.

40 41 98 **Methods**

42 43 44 99 **Study setting, participants and sample collection**

45
46
47 100 The study was conducted at the Royal College of Surgeons in Ireland (RCSI) from
48
49 101 December 2014 – January 2016. Nasal swabs (eSwab Copan®), Italy) were collected
50
51 102 anonymously from undergraduate medical students. Eligible students were those
52
53 103 attending the RCSI medical centre to submit a swab for mandatory MRSA screening in
54
55 104 the week before they began their clinical attachments. In total 444/450 eligible medical

1
2
3 105 students (250 (56.3%) male, 194 (43.7%) female) participated in this study. All
4
5 106 participants reported no previous hospital contact in the six weeks prior to recruitment.
6
7 107 The student volunteers were from the second year of the undergraduate medical
8
9 108 programme and as such, all participants were domiciled in Ireland for a minimum of two
10
11 109 years prior to recruitment. Data was collected anonymously from each participant,
12
13 110 including age range, region of origin and previous healthcare contact. Ethical approval
14
15 111 (approval number REC949) was obtained from the Institute's Ethics Committee and
16
17 112 informed consent was obtained from each participant.

113 **Sample preparation**

114 Swabs were processed to recover *S. aureus* (including MRSA) and CoNS using a
115 modification of a published method[11]. Swabs were enriched in brain heart infusion
116 (BHI) supplemented with 6% (w/v) NaCl for 24 h at 37°C followed by further enrichment
117 in mannitol salt broth for 24 h at 37°C. The enriched culture was diluted 1/1000 and
118 100 µl was spread onto SaSelect agar (Bio-Rad®, Hercules, CA, USA). Plates that
119 yielded pink/orange colonies (presumptive *S. aureus*) were inspected for growth of
120 colonies of relevant CoNS (e.g. light pink colonies of various sizes, presumptive *S.*
121 *epidermidis*; white/yellow colonies, *S. haemolyticus*, *S. hominis*, *S. capitis*, *S. warneri*,
122 *S. caprae*, *S. lugdunensis*). Presumptive CoNS species and *S. aureus* were sub-
123 cultured from these plates onto Columbia blood agar (CBA) and identified by matrix-
124 assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF)
125 using a MALDI Biotyper (Microflex LT, Bruker). Matched isolates (where *S. aureus* and
126 a CoNS species were recovered from the same swab) were cryopreserved and stored
127 at -20°C (Protect™ bacterial preserver beads (Technical Service Consultants, UK).

128

129 **Characterisation of *S. aureus* and CoNS isolates**

130 Genomic DNA from *S. aureus* and CoNS isolates was extracted using enzymatic lysis
131 using the buffers and solutions provided with the *S. aureus* Genotyping Kit 2.0 (Alere

1
2
3 132 Technologies GmbH, Jena, Germany) and a DNeasy® Blood and Tissue kit (Qiagen,
4
5 133 Crawley, UK). Genetic characterisation of isolates was undertaken by DNA microarray
6
7 134 profiling using the *S. aureus* Genotyping Kit 2.0 as described previously [12 13]. The kit
8
9 135 detects 333 gene targets including staphylococcal antimicrobial-resistance, virulence,
10
11 136 SCC*mec* and ACME-*arc* genes and assigns *S. aureus* isolates to multilocus sequence
12
13 137 type (ST) or clonal complexes (CC)s. MRSA phenotype was confirmed in *S. aureus*
14
15 138 and CoNS isolates positive for *mecA* by growth of pink (*S. aureus*) or colorless/white
16
17 139 (CoNS) colonies on MRSASelect agar (Bio-Rad®, Hercules, CA, USA). When required,
18
19 140 confirmation of carriage of *fusC*, *fusB* and *tst1* was confirmed by PCR using the
20
21 141 primers and conditions described by O'Neill et. al. [14] and Chen et. al. [15]
22
23 142

24 25 143 **Fusidic acid susceptibility testing**

26
27 144 Fusidic acid MICs were determined by ETEST® (bioMérieux, Marcy-l'Étoile, France)
28
29 145 according to manufacturer's instructions. Thirty *S. aureus* and CoNS isolates
30
31 146 harbouring fusidic acid resistance genes (*fusC* or *fusB*) were sub-cultured twice before
32
33 147 testing. Results were interpreted according to EUCAST (<http://www.eucast.org>,
34
35 148 assessed May 2015) susceptibility criteria.
36
37

38 39 149 **Statistical analyses**

40
41 150 Fisher's exact test was used to analyze categorical variables (prevalence of genes)
42
43 151 using GraphPad QuickCalcs on-line software. The significance of differences between
44
45 152 groups was expressed as two-tailed p-values, *p* values of ≤ 0.05 were considered
46
47 153 statistically significant.
48
49 154

50 51 155 **Results**

52 53 54 156 **Nasal carriage of staphylococcal species and regional distribution**

1
2
3 157 Thirty-one percent (137/444) of students were positive for nasal carriage of *S. aureus*
4
5 158 of whom 6.5 % (9/137) were MRSA. Eighty-seven percent (386/444) of students were
6
7 159 positive for nasal carriage of CoNS (*S. epidermidis* (82% 364/444), *S. haemolyticus*
8
9 160 (3% 14/444) or *S. saprophyticus* (2% 8/444). Methicillin-resistant (MR)-CoNS were
10
11 161 investigated in the *S. aureus*-positive cohort only of which 13.1 % (18/137) were MR-
12
13 162 CoNS. One student exhibited co-carriage of MRSA and MR-CoNS. The geographical
14
15 163 region of origin of students harbouring *S. aureus* and CoNS is shown in Figure 1. The
16
17 164 Middle East, Europe and North America accounted for 68.6% of *S. aureus* carriers. For
18
19 165 regions represented by ≥ 12 participants, the rate of nasal carriage of *S. aureus* varied
20
21 166 geographically between 17 % (South East Asia) and 44 % (Africa).

22 23 167 **Clonal lineages among *S. aureus* isolates**

24
25
26 168 The ST or CC distribution among 137 *S. aureus* isolates is shown in Figure 2. Isolates
27
28 169 belonged to a variety of CCs with 46/137 (33.5%) assigned to internationally
29
30 170 disseminated CC5, CC8, CC22, CC30, CC45. A further 24/137 (17.5%) isolates
31
32 171 belonged to CC1, CC59, CC88 or CC398.

33 34 35 36 37 173 **SCCmec types and fusidic acid resistance among *S. aureus* and CoNS**

38
39
40 174 Of the 333 staphylococcal genes detected by the microarray, the two most prevalent
41
42 175 antibiotic resistance genes among nasal staphylococci were those encoding resistance
43
44 176 to β -lactams and fusidic acid. The most common SCCmec type among nasal MRSA
45
46 177 (n=9) and MR-CoNS (n=18) was SCCmec type IV. The nine MRSA isolates belonged
47
48 178 to ST59-MRSA-IV (6/9) and CC1-MRSA-V-SCCfus (3/9). Among 18 MR-CoNS
49
50 179 identified (17 *S. epidermidis* and 1 *S. saprophyticus*), half harboured SCCmec type IV
51
52 180 (8 *S. epidermidis* and the single *S. saprophyticus*). SCCmec types II, V and VII were
53
54 181 identified in three, five and one of the remaining *S. epidermidis* isolates, respectively.

1
2
3 182 Isolates from the one individual who exhibited nasal co-carriage of MRSA and MR-
4
5 183 CoNS (*S. epidermidis*) both harboured SCCmec type IV (Table 1).
6

7 184 In addition to the three CC1-MRSA-V isolates that carried SCCfus, the fusidic acid
8
9 185 resistance genes *fusC* and *fusB* were identified in 28/128 (21.8%) and 2/128 (1.5%) of
10
11 186 methicillin-susceptible *S. aureus* (MSSA) isolates, respectively. Ten of the 28 *fusC*-
12
13 187 positive MSSA isolates belonged to CC1-MSSA-SCCfus and 18 were CC88-MSSA. All
14
15 188 10 CC1-MSSA-SCCfus isolates harboured a combination of SCCfus with the *ccr* genes
16
17 189 *ccrA1 ccrB-1*. The two *fusB* positive isolates belonged to CC5-MSSA and CC8-MSSA
18
19 190 (Table 1). Among MR-CoNS, 27.7 % (5/18) *S. epidermidis* isolates carried *fusC* (two of
20
21 191 them also carried *ccr* genes *ccrA1 ccrB-1*) and 50 % *fusB* (9/18, eight *S. epidermidis*
22
23 192 and the one *S. saprophyticus*). Among methicillin susceptible CoNS isolates, the *fusC*
24
25 193 and *fusB* genes were identified in 20/119 (16.8%, 18 *S. epidermidis* and two *S.*
26
27 194 *saprophyticus*) and 18/119 (15.1%, all *S. epidermidis*), respectively. One participant
28
29 195 had nasal co-carriage of *fusC*-positive *S. aureus* (CC88-MSSA) and CoNS (*S.*
30
31 196 *epidermidis*).
32

33
34 197 All SCCmec positive staphylococci were confirmed to have an MRSA/MR-
35
36 198 CoNS phenotype. However, there was poor correlation between *fusC/fusB* carriage
37
38 199 and phenotypic fusidic acid resistance. Fusidic acid MICs for all *fusC* or *fusB*-positive
39
40 200 *S. aureus* and CoNS isolates are shown in Table 2. Phenotypic fusidic acid resistance
41
42 201 was confirmed (based on EUCAST breakpoints, MICs ≥ 1 $\mu\text{g/ml}$) in 23/32 (71.8%) *S.*
43
44 202 *aureus* and 20/38 (52.6%) CoNS nasal isolates harbouring either *fusC* or *fusB* (DNA
45
46 203 microarray result confirmed by PCR). Eight nasal isolates (three *S. aureus*, five *S.*
47
48 204 *epidermidis*) positive for *fusB* exhibited high level fusidic acid resistance (MIC \geq
49
50 205 32 $\mu\text{g/ml}$). Fusidic acid resistance was inducible in a further three *S. aureus* and seven
51
52 206 *S. epidermidis* isolates following incubation with 0.01 $\mu\text{g/ml}$ fusidic acid BHI agar.
53

54 207 **Other notable antimicrobial resistance genes among nasal *S. aureus* and CoNS**
55
56
57
58
59
60

1
2
3 208 Apart from SCC*mec* element and *fus* genes, other antimicrobial genes detected among
4
5 209 staphylococcal nasal flora were identified by DNA microarray. Tetracycline resistance
6
7 210 genes, *tet*(K) or *tet*(M), were detected in 13/137 (9.5 %) of *S. aureus* isolates and 6/137
8
9 211 (4.3%) of the CoNS isolates. The quaternary ammonium compound resistance genes
10
11 212 (*qacA/qacC*), encoding antiseptic resistance, were significantly more prevalent among
12
13 213 CoNS isolates compared to *S. aureus* isolates (29/137 (21.2%) Vs 2/137 (1.4%),
14
15 214 $p < 0.0001$). Significantly more CoNS than *S. aureus* isolates carried *ileS2* encoding
16
17 215 high-level mupirocin resistance (11/137 (8%) vs 1/137 (0.72%), $p < 0.01$). However,
18
19 216 none of these genes were common to *S. aureus*/CoNS pairs recovered from the same
20
21 217 individual. The β -lactamase genes were abundant among *S. aureus* and CoNS; *blaZ*
22
23 218 was present in 101/137 (73.72%) *S. aureus* isolates and 92/137 (67.1%) CoNS isolate
24
25 219 and in 74/137 (54%) of individuals, these genes were common to *S. aureus*/CoNS
26
27 220 pairs from the same nares. A summary of the antibiotic resistance genes found among
28
29 221 *S. aureus* and CoNS is shown in Table 1. The staphylococcal isolates were negative
30
31 222 for all other antibiotic resistance genes detected by the microarray.

223 **Virulence genes among nasal *S. aureus* and CoNS**

32
33
34
35 224 A single isolate, CC30-MSSA, was positive for the Pantone-Valentine leucocidin genes
36
37 225 (*lukF/S-PV*). Among nasal staphylococci, ACME-*arc* was significantly associated with
38
39 226 CoNS compared to *S. aureus* (44/137 (32.1%) Vs 1/137 (0.7%)), $p < 0.0001$. The toxic
40
41 227 shock syndrome toxin gene *tst1* was identified in 33/137 (24.1%) nasal *S. aureus*
42
43 228 isolates. Unusually, DNA microarray identified *tst1* in two *S. epidermidis* isolates and
44
45 229 this was confirmed by PCR. ACME-*arc* was common to *S. aureus*/CoNS recovered
46
47 230 from the nares in one individual only. One hundred and two (74.4%) *S. aureus* isolates
48
49 231 encoded one or more enterotoxin genes. The enterotoxin gene cluster (*egc*), containing
50
51 232 *seg*, *sei*, *sem*, *sen*, *seo*, *seu*) was the most prevalent (48/102, 47%) followed by *seq/k*
52
53 233 (13/102, 12.7 %) and *sec/I* (7/102, 6.8 %). Staphylococcal isolates were negative for all
54
55 234 other toxin genes detected by the microarray.

235

236 **Discussion**

237 Studies of staphylococcal carriage and epidemiology among the healthy
238 population in the absence of significant antibiotic pressure are important in identifying
239 the potential for pathogenic evolution. To our knowledge, this is the first study to co-
240 investigate CoNS and *S. aureus* when recovered together from the nares of healthy
241 pre-clinical medical students. Our study revealed that, apart from the *bla* genes, which
242 are abundant among staphylococci, the rates of co-carriage of antibiotic resistance
243 genes in paired *S. aureus*/CoNS from the same individual were low in the community
244 setting at <1%. Rates of simultaneous carriage of antimicrobial resistance among nasal
245 staphylococci are likely to be higher under selective antibiotic pressure but few studies
246 have investigated this among patients. One small study of hospitalized patients with
247 nasal carriage of *S. aureus* and CoNS reported a rate of 12.5 % patients carrying
248 MRSA and MR-CoNS [16]. However, the authors reported only two cases where
249 simultaneous carriage of MR-CoNS and MRSA was detected and the strains involved
250 carried different SCC*mec* types. Despite negligible detection of co-species nasal carriage
251 of these genes in medical students prior to healthcare exposure, based on
252 antimicrobial resistance gene carriage by CoNS from this cohort, there is significant
253 potential for mobilisation of genes to *S. aureus* that may enhance its pathogenic
254 potential in the healthcare setting.

255 DNA microarray analyses revealed carriage of SCC*mec*, *fusC*, *fusB*, *ileS2*,
256 *qacA/qacC* and ACME-*arc* among colonising nasal staphylococci in individuals with no
257 previous healthcare exposure with greater prevalence among CoNS than *S. aureus*.
258 This pattern among pre-clinical medical students, supports CoNS as a reservoir with
259 potential to subsequently accelerate antimicrobial resistance and pathogenicity among
260 colonizing *S. aureus* in clinical environments under antibiotic selective pressure [17-
261 19].

1
2
3 262 Despite considerable geographical distribution of the participants in this study, a
4
5 263 *S. aureus* nasal carriage rate in the community of 30.8% was recorded. In this study,
6
7 264 CC30, CC88 and CC8 were the most prevalent clones identified among nasal *S.*
8
9 265 *aureus*. CC30 is among the internationally disseminated clones in which *SCCmec* has
10
11 266 been acquired and is a successful colonising strain, reported among HA and CA-
12
13 267 MRSA. CC88 is frequently isolated in Australia but in our study the geographical
14
15 268 background of isolates was mixed (including Middle East, Europe, South East Asia and
16
17 269 Central America). CC8 is associated with MRSA infection and is globally disseminated
18
19 270 [20]. Two CC/ST types were detected among MRSA recovered from healthy medical
20
21 271 students in this study, ST59-MRSA-IV and CC1-MRSA-V+*SCCfusC*. ST59 (WA-
22
23 272 MRSA-73) is a sporadic Australian strain and apart from PVL-negativity, is
24
25 273 indistinguishable from USA1000 [21]. In this study the geographical background of
26
27 274 these isolates was wide (Middle East, North America and South East Asia).

28
29 275 The identification of a significant reservoir of antibiotic resistance among
30
31 276 medical students prior to healthcare exposure in subsequent clinical years, highlights
32
33 277 the need for effective infection prevention and control policies in relation to hand
34
35 278 hygiene and surveillance. In the absence of antibiotic selective pressure, the
36
37 279 colonising MRSA rate appears relatively stable and in this study was 2 % (9/444),
38
39 280 similar to rates reported elsewhere [22]. However, a previous study among medical
40
41 281 interns in China reported a nasal MRSA rate of 9.4% likely reflecting exposure to the
42
43 282 healthcare environment [23]. Prevalence rates of MR-CoNS in recent community-
44
45 283 based surveys are variable but rates of 16.5% [18] and 17.2% [24] are reported in
46
47 284 similar cohorts to this study where, of those colonised with *S. aureus*, 13.1% carried
48
49 285 MR-CoNS. *SCCmec* type IV, the smallest of the *SCCmec* elements, was the most
50
51 286 prevalent type among MRSA and MR-CoNS here (66.6% and 50%). *SCCmec* IV has
52
53 287 been detected in approximately 40% of methicillin-resistant *S. epidermidis* identified in
54
55 288 humans [25]. However, in this study *SCCmec* type V was also represented among
56
57
58
59
60

1
2
3 289 MRSA and MR-CoNS. While only one individual was colonised with MRSA and MR-
4
5 290 CoNS in this study (both SCCmec type IV), the preponderance of this SCCmec
6
7 291 element in nasal MRSA and MR-CoNS suggests the potential for *mecA* gene transfer
8
9 292 among these species even in the absence of selective pressure. The small size of this
10
11 293 element, which has a low fitness cost, may enhance its dissemination potential. [26]

12
13 294 Fusidic acid resistance among *S. aureus* from healthy carriers in nine European
14
15 295 countries in 2014 was reported to be <10 % [27]. However, we found a prevalence of
16
17 296 21.9% of *fusC/fusB* genes among healthy carriers. Fusidic acid resistance appears to
18
19 297 correlate with increased use of this agent. For example, in New Zealand, where it is
20
21 298 used as a first-line empiric agent for topical treatment of impetigo, prevalence rates of
22
23 299 resistance in community *S. aureus* isolates increased from 17% in 1999 to 29% in 2013
24
25 300 [28]. In Europe, fusidic acid is combined with β -lactams for the treatment of
26
27 301 staphylococcal bacteraemia, endocarditis, and osteomyelitis [29] and is used widely in
28
29 302 the community for SSTIs. A 2010 study of fusidic acid resistance among *S. aureus*
30
31 303 clinical isolates showed Greece and Ireland to have the highest rates (52.5 and 19.9%)
32
33 304 [30]. SCCfus has been identified in the CC1 background and more recently, in other
34
35 305 lineages such as ST239 and ST779 [31-33]. As highlighted here in the absence of
36
37 306 significant antibiotic pressure in the community, it appears that this element is
38
39 307 associated with MRSA and MSSA in the CC1 background. This genetic platform,
40
41 308 particularly when associated with SCCmec, may enable the transfer of multi-drug
42
43 309 resistance on a single mobile element. The use of fusidic acid is un-regulated in some
44
45 310 countries and hence it may be used inappropriately in a community setting (for
46
47 311 example in short or discontinuous doses). Inappropriate use of fusidic acid may
48
49 312 therefore favour co-selection of methicillin-resistance among *S. aureus*. In addition, in
50
51 313 this study, 14/18 (77%) of MR-CoNS were positive for *fusC* or *fusB*. This association of
52
53 314 resistances among the resident flora may provide further opportunity for dissemination
54
55 315 of MRSA driven by fusidic acid selective pressure. Interestingly a positive correlation

1
2
3 316 between carriage of *fusC/fusB* and phenotypic resistance was observed in only 71.9 %
4 317 and 53.6 % of *S. aureus* and CoNS respectively. However, induction of gene
5
6 318 expression with fusidic acid pre-incubation gave better correlation (82.2% and 76.3 %
7
8 319 correlation).

10
11 320 There were limitations to this study, which included; a single centred, relatively
12
13 321 small study. CoNS was investigated only in those co-colonised with *S. aureus*. CCs
14
15 322 and STs were determined only among *S. aureus* as the high rate of genetic
16
17 323 recombination among CoNS makes strain typing unreliable. Although the microarray
18
19 324 system used is reported effective for staphylococcal species other than *S. aureus* [34],
20
21 325 some gene targets may be heterologous among staphylococci leading to false
22
23 326 negatives. The study design did not facilitate follow-up of this cohort during clinical
24
25 327 training which may have revealed further changes in gene carriage among colonising
26
27 328 staphylococci. However, the multi-national origin of the student body in our institution
28
29 329 facilitated analysis of a relatively broad geographic cohort in a single study and
30
31 330 emphasises the role that importation plays in *S. aureus* epidemiology. Unlike other
32
33 331 studies of staphylococci in the healthy human nares, pairs of staphylococcal species
34
35 332 originating from the same individual were investigated here for their resistance and
36
37 333 virulence traits. These data support a low rate of transfer of antibiotic resistance
38
39 334 between colonising staphylococcal species in the absence of healthcare contact.
40
41 335 However, it is concerning that similar *SCCmec* and *SCCfusC* types, in addition to *ileS2*,
42
43 336 *qacA/qacB* and ACME are carried among CoNS and *S. aureus* in healthy individuals
44
45 337 who will have subsequent roles in healthcare provision. Given the increasing
46
47 338 emergence of HA-MRSA with features of community strains, further mobilisation of
48
49 339 these elements under selective antibiotic pressure may enhance the transmission and
50
51 340 success of *S. aureus* in the healthcare environment.

52
53 341

54
55
56 342 **Funding statement**

1
2
3 343 This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível
4
5 344 Superior (CAPES) Brazil, Science without Borders Program, Award 9172-13-0. The
6
7 345 funder had no involvement in study design, data collection, analysis or interpretation or
8
9 346 any other aspect of the submitted work.

10
11 347

12 13 14 348 **Competing Interests Statement**

15
16 349 HH has received funding from Pfizer and Astellas outside the relevance of the
17
18 350 submitted work. All other authors report no competing interests.

19
20
21 351

22 23 352 **Contributorship statement**

24
25
26 353 PEB and DFH recruited students to the study, PEB conducted the laboratory work and
27
28 354 drafted the manuscript. DFH and HH conceived of the study and contributed to study
29
30 355 design. AS, PK and DC provided critical data interpretation and revised the drafted
31
32 356 work. All authors contributed to the final approved draft.

33
34
35 357

36
37 358 **Data Sharing Statement** All data for these analyses are included in the manuscript.

38
39 359 No additional data are available.

40
41
42 360

43 44 361 **Acknowledgements**

45
46
47 362 We acknowledge the support of the Mercer's Medical Centre, RCSI and Ms. Helen
48
49 363 Barry, Chief Medical Scientist, St. James' Hospital Dublin.

50
51
52 364

53 54 365 **Figure Legends**

1
2
3 366 **Figure 1.** The geographical origin of 444 medical students from whom a nasal swab
4
5 367 was collected (dark grey bars) and the proportion of participants with nasal co-carriage
6
7 368 of *S. aureus* and CoNS (light grey bars).
8

9 369

10
11
12 370 **Figure 2.** Genotypic diversity of 137 *S. aureus* nasal isolates using DNA microarray
13
14 371 analysis, including 128 MSSA (grey bars) and 9 MRSA (white bars). Bold lettering
15
16 372 indicates internationally disseminated clones into which SCCmec can integrate.
17
18 373 CC=clonal complex

19
20 374

21
22
23 375

24
25 376
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

377 Table 1. Resistance/virulence genes detected among *S. aureus* and CoNS nasal isolates.

Detected Gene(s)	Phenotypic resistance/trait	No. isolates positive		<i>P</i> value	No. <i>S. aureus</i> /CoNS pairs positive (n)
		<i>S. aureus</i> n (%)	CoNS n (%)		
		n=137	n=137		
<i>Antibiotic resistance gene</i>					
<i>blaZ</i>	β-lactam	101 (73.7)	92 (67.1)	0.289	74
<i>fusB</i>	Fusidic acid	2 (1.5)	18 (13.3)	0.0002*	0
<i>fusC^a</i>	Fusidic acid	30 (21.9)	20 (14.5)	0.159	2
<i>mecA</i>	Methicillin	9 (6.5)	18 (13.1)	0.103	1
<i>ileS2</i>	Mupirocin	1 (0.7)	11 (8.0)	0.005*	1
<i>qacA</i> and <i>qacC</i>	Quaternary ammonium salts	3 (2.2)	29 (21.2)	<0.0001*	0
<i>tet(K)</i> and <i>tet(M)</i>	Tetracycline	13 (9.5)	6 (4.4)	0.152	0
<i>erm(C)</i>	Macrolide/lincosamide	6 (4.3)	5 (3.6)	1.000	0
<i>msr(A)</i>	Macrolide	2 (1.45)	15 (10.9)	0.002*	1
<i>mph(C)</i>	Macrolide	0	15 (10.9)	<0.0001*	0
<i>dfpS1</i>	Trimethoprim	0	19 (13.8)	<0.0001*	0

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

<i>vga</i>	Streptogramin A	1 (0.7)	6 (4.3)	0.120	0
<i>Virulence</i>					
ACME- <i>arc</i>	pH tolerance	1 (0.7)	44 (32.1)	<0.0001*	1
<i>tst1^b</i>	Toxic shock toxin	33 (24.1)	2 (1.5)	<0.0001*	0

378 ^aassociated with SCC element, (*ccrA1* and *ccrB*-) in 13/137 *S. aureus*. ^b*tst1* confirmed by PCR. ACME = Arginine Catabolite Mobile Element. * indicates a
 379 statistically significant result by Fisher's exact test.

380

For peer review only

1
2
3
4
5
6 3817
8 3829
10
11 383 Table 2. Fusidic acid MICs for *S. aureus* and CoNS12
13
14 384

MIC Interpretation ^a	MIC ≤ 1 µg/ml	MIC ≥ 1 µg/ml	MIC ≥ 32 µg/ml
	S	R	HR
	n (%)	n(%)	n (%)
<i>S. aureus</i> (n = 32)	9 (28.1)	20 (62.5)	3 (9.3)
CoNS (n = 38)	18 (47.4)	15 (39.5)	5 (13.2)

15
16
17
18
19
20
21
22
23
24
25
26 385 ^aInterpretation based on The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone
27
28 386 diameters. Version 7.1, 2017. <http://www.eucast.org>, S = Susceptible, R = Resistant, HR = high level resistant

29
30
31 387

388 **References**

389

- 390 1. Fluit AC, Carpaij N, Majoor EA, et al. Shared reservoir of *ccrB* gene sequences
391 between coagulase-negative staphylococci and methicillin-resistant
392 *Staphylococcus aureus*. *The Journal of antimicrobial chemotherapy*
393 2013;**68**(8):1707-13 doi: 10.1093/jac/dkt121[published Online First: Epub
394 Date]].
- 395 2. Shore AC, Coleman DC. Staphylococcal cassette chromosome *mec*: recent
396 advances and new insights. *International journal of medical microbiology : IJMM*
397 2013;**303**(6-7):350-9 doi: 10.1016/j.ijmm.2013.02.002[published Online First:
398 Epub Date]].
- 399 3. Hung WC, Chen HJ, Lin YT, et al. Skin Commensal Staphylococci May Act as
400 Reservoir for Fusidic Acid Resistance Genes. *PloS one* 2015;**10**(11):e0143106
401 doi: 10.1371/journal.pone.0143106[published Online First: Epub Date]].
- 402 4. Ellington MJ, Reuter S, Harris SR, et al. Emergent and evolving antimicrobial
403 resistance cassettes in community-associated fusidic acid and methicillin-
404 resistant *Staphylococcus aureus*. *International journal of antimicrobial agents*
405 2015;**45**(5):477-84 doi: 10.1016/j.ijantimicag.2015.01.009[published Online
406 First: Epub Date]].
- 407 5. Baines SL, Howden BP, Heffernan H, et al. Rapid Emergence and Evolution of
408 *Staphylococcus aureus* Clones Harboring *fusC*-Containing Staphylococcal
409 Cassette Chromosome Elements. 2016;**60**(4):2359-65 doi: 10.1128/aac.03020-
410 15[published Online First: Epub Date]].
- 411 6. Miragaia M, de Lencastre H, Perdreau-Remington F, et al. Genetic diversity of
412 arginine catabolic mobile element in *Staphylococcus epidermidis*. *PloS one*
413 2009;**4**(11):e7722 doi: 10.1371/journal.pone.0007722[published Online First:
414 Epub Date]].

- 1
2
3 415 7. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an
4
5 416 epidemic clone of community-acquired methicillin-resistant *Staphylococcus*
6
7 417 *aureus*. *Lancet* (London, England) 2006;**367**(9512):731-9 doi: 10.1016/s0140-
8
9 418 6736(06)68231-7[published Online First: Epub Date]].
- 10
11 419 8. Gonzalez-Dominguez M, Seral C, Potel C, et al. Genotypic and phenotypic
12
13 420 characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) clones
14
15 421 with high-level mupirocin resistance. *Diagn Microbiol Infect Dis* 2016;**85**(2):213-
16
17 422 7 doi: 10.1016/j.diagmicrobio.2016.02.021[published Online First: Epub Date]].
- 18
19 423 9. Bathoorn E, Hetem DJ, Alphenaar J, et al. Emergence of high-level mupirocin
20
21 424 resistance in coagulase-negative staphylococci associated with increased
22
23 425 short-term mupirocin use. *J Clin Microbiol* 2012;**50**(9):2947-50 doi:
24
25 426 10.1128/jcm.00302-12[published Online First: Epub Date]].
- 26
27 427 10. Earls MR, Kinnevey PM, Brennan GI, et al. The recent emergence in hospitals of
28
29 428 multidrug-resistant community-associated sequence type 1 and spa type t127
30
31 429 methicillin-resistant *Staphylococcus aureus* investigated by whole-genome
32
33 430 sequencing: Implications for screening. 2017;**12**(4):e0175542 doi:
34
35 431 10.1371/journal.pone.0175542[published Online First: Epub Date]].
- 36
37 432 11. Huber H, Giezendanner N, Stephan R, et al. Genotypes, antibiotic resistance
38
39 433 profiles and microarray-based characterization of methicillin-resistant
40
41 434 *Staphylococcus aureus* strains isolated from livestock and veterinarians in
42
43 435 Switzerland. *Zoonoses and public health* 2011;**58**(5):343-9 doi: 10.1111/j.1863-
44
45 436 2378.2010.01353.x[published Online First: Epub Date]].
- 46
47 437 12. Monecke S, Jatzwauk L, Weber S, et al. DNA microarray-based genotyping of
48
49 438 methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. *Clin*
50
51 439 *Microbiol Infect* 2008;**14**(6):534-45 doi: CLM1986 [pii]
52
53 440 10.1111/j.1469-0691.2008.01986.x [doi][published Online First: Epub Date]].
54
55
56
57
58
59
60

- 1
2
3 441 13. Monecke S, Slickers P, Ehricht R. Assignment of *Staphylococcus aureus* isolates to
4
5 442 clonal complexes based on microarray analysis and pattern recognition. *FEMS*
6
7 443 *Immunol Med Microbiol* 2008;**53**(2):237-51 doi: FIM426 [pii]
8
9 444 10.1111/j.1574-695X.2008.00426.x [doi][published Online First: Epub Date]].
10
11 445 14. O'Neill AJ, Larsen AR, Henriksen AS, et al. A fusidic acid-resistant epidemic strain
12
13 446 of *Staphylococcus aureus* carries the fusB determinant, whereas fusA
14
15 447 mutations are prevalent in other resistant isolates. *Antimicrobial agents and*
16
17 448 *chemotherapy* 2004;**48**(9):3594-7 doi: 10.1128/aac.48.9.3594-
18
19 449 3597.2004[published Online First: Epub Date]].
20
21 450 15. Chen HJ, Hung WC, Tseng SP, et al. Fusidic acid resistance determinants in
22
23 451 *Staphylococcus aureus* clinical isolates. *Antimicrobial agents and*
24
25 452 *chemotherapy* 2010;**54**(12):4985-91 doi: 10.1128/aac.00523-10[published
26
27 453 Online First: Epub Date]].
28
29 454 16. Faria NA, Conceicao T, Miragaia M, et al. Nasal carriage of methicillin resistant
30
31 455 staphylococci. *Microb Drug Resist* 2014;**20**(2):108-17 doi:
32
33 456 10.1089/mdr.2013.0197[published Online First: Epub Date]].
34
35 457 17. Jamaluddin TZ, Kuwahara-Arai K, Hisata K, et al. Extreme genetic diversity of
36
37 458 methicillin-resistant *Staphylococcus epidermidis* strains disseminated among
38
39 459 healthy Japanese children. *Journal of clinical microbiology* 2008;**46**(11):3778-83
40
41 460 doi: 10.1128/jcm.02262-07[published Online First: Epub Date]].
42
43 461 18. Barbier F, Ruppe E, Hernandez D, et al. Methicillin-resistant coagulase-negative
44
45 462 staphylococci in the community: high homology of SCCmec IVa between
46
47 463 *Staphylococcus epidermidis* and major clones of methicillin-resistant
48
49 464 *Staphylococcus aureus*. *J Infect Dis* 2010;**202**(2):270-81 doi: 10.1086/653483
50
51 465 [doi][published Online First: Epub Date]].
52
53 466 19. Iravani Mohammad Abadi M, Moniri R, Khorshidi A, et al. Molecular Characteristics
54
55 467 of Nasal Carriage Methicillin-Resistant Coagulase Negative Staphylococci in
56
57
58
59
60

- 1
2
3 468 School Students. Jundishapur journal of microbiology 2015;**8**(6):e18591 doi:
4
5 469 10.5812/jjm.18591v2[published Online First: Epub Date]].
6
7 470 20. Jimenez JN, Ocampo AM, Vanegas JM, et al. CC8 MRSA strains harboring
8
9 471 SCCmec type IVc are predominant in Colombian hospitals. PloS one
10
11 472 2012;**7**(6):e38576 doi: 10.1371/journal.pone.0038576[published Online First:
12
13 473 Epub Date]].
14
15 474 21. Monecke S, Coombs G, Shore AC, et al. A field guide to pandemic, epidemic and
16
17 475 sporadic clones of methicillin-resistant *Staphylococcus aureus*. PloS one
18
19 476 2011;**6**(4):e17936 doi: 10.1371/journal.pone.0017936[published Online First:
20
21 477 Epub Date]].
22
23 478 22. Abroo S, Hosseini Jazani N, Sharifi Y. Methicillin-resistant *Staphylococcus aureus*
24
25 479 nasal carriage between healthy students of medical and nonmedical
26
27 480 universities. American journal of infection control 2017;**45**(7):709-12 doi:
28
29 481 10.1016/j.ajic.2017.02.034[published Online First: Epub Date]].
30
31 482 23. Ma XX, Sun DD, Wang S, et al. Nasal carriage of methicillin-resistant
32
33 483 *Staphylococcus aureus* among preclinical medical students: epidemiologic and
34
35 484 molecular characteristics of methicillin-resistant *S. aureus* clones. Diagnostic
36
37 485 microbiology and infectious disease 2011;**70**(1):22-30 doi:
38
39 486 10.1016/j.diagmicrobio.2010.12.004[published Online First: Epub Date]].
40
41 487 24. Du X, Zhu Y, Song Y, et al. Molecular analysis of *Staphylococcus epidermidis*
42
43 488 strains isolated from community and hospital environments in China. PloS one
44
45 489 2013;**8**(5):e62742 doi: 10.1371/journal.pone.0062742[published Online First:
46
47 490 Epub Date]].
48
49 491 25. Miragaia M, Thomas JC, Couto I, et al. Inferring a population structure for
50
51 492 *Staphylococcus epidermidis* from multilocus sequence typing data. Journal of
52
53 493 bacteriology 2007;**189**(6):2540-52 doi: 10.1128/jb.01484-06[published Online
54
55 494 First: Epub Date]].
56
57
58
59
60

- 1
2
3 495 26. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant
4
5 496 Staphylococcus aureus (MRSA) strains replacing traditional nosocomial MRSA
6
7 497 strains? *Clinical infectious diseases : an official publication of the Infectious*
8
9 498 *Diseases Society of America* 2008;**46**(6):787-94 doi: 10.1086/528716[published
10
11 499 Online First: Epub Date]].
- 12
13 500 27. den Heijer CD, van Bijnen EM, Paget WJ, et al. Fusidic acid resistance in
14
15 501 Staphylococcus aureus nasal carriage strains in nine European countries.
16
17 502 *Future Microbiol* 2014;**9**(6):737-45 doi: 10.2217/fmb.14.36[published Online
18
19 503 First: Epub Date]].
- 20
21 504 28. Williamson DA, Monecke S, Heffernan H, et al. High usage of topical fusidic acid
22
23 505 and rapid clonal expansion of fusidic acid-resistant Staphylococcus aureus: a
24
25 506 cautionary tale. *Clinical infectious diseases : an official publication of the*
26
27 507 *Infectious Diseases Society of America* 2014;**59**(10):1451-4 doi:
28
29 508 10.1093/cid/ciu658[published Online First: Epub Date]].
- 30
31 509 29. Whitby M. Fusidic acid in septicaemia and endocarditis. *International journal of*
32
33 510 *antimicrobial agents* 1999;**12**:S17-S22 doi: <http://dx.doi.org/10.1016/S0924->
34
35 511 [8579\(98\)00070-3](http://dx.doi.org/10.1016/S0924-8579(98)00070-3)[published Online First: Epub Date]].
- 36
37 512 30. Castanheira M, Watters AA, Mendes RE, et al. Occurrence and molecular
38
39 513 characterization of fusidic acid resistance mechanisms among Staphylococcus
40
41 514 spp. from European countries (2008). *The Journal of antimicrobial*
42
43 515 *chemotherapy* 2010;**65**(7):1353-8 doi: 10.1093/jac/dkq094[published Online
44
45 516 First: Epub Date]].
- 46
47 517 31. Holden MT, Feil EJ, Lindsay JA, et al. Complete genomes of two clinical
48
49 518 Staphylococcus aureus strains: evidence for the rapid evolution of virulence and
50
51 519 drug resistance. *Proc Natl Acad Sci U S A* 2004;**101**(26):9786-91 doi:
52
53 520 10.1073/pnas.0402521101[published Online First: Epub Date]].
- 54
55 521 32. Kinnevey PM, Shore AC, Brennan GI, et al. Emergence of sequence type 779
56
57 522 methicillin-resistant Staphylococcus aureus harboring a novel pseudo

1
2
3 523 staphylococcal cassette chromosome mec (SCCmec)-SCC-SCCCRISPR
4
5 524 composite element in Irish hospitals. *Antimicrobial agents and chemotherapy*
6
7 525 2013;**57**(1):524-31 doi: 10.1128/aac.01689-12[published Online First: Epub
8
9 526 Date]].

10
11 527 33. Lin YT, Tsai JC, Chen HJ, et al. A novel staphylococcal cassette chromosomal
12
13 528 element, SCCfusC, carrying fusC and speG in fusidic acid-resistant methicillin-
14
15 529 resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*
16
17 530 2014;**58**(2):1224-7 doi: 10.1128/aac.01772-13[published Online First: Epub
18
19 531 Date]].

20
21 532 34. Argudin MA, Vanderhaeghen W, Butaye P. Diversity of antimicrobial resistance and
22
23 533 virulence genes in methicillin-resistant non-*Staphylococcus aureus*
24
25 534 staphylococci from veal calves. *Research in veterinary science* 2015;**99**:10-6
26
27 535 doi: 10.1016/j.rvsc.2015.01.004[published Online First: Epub Date]].
28

29
30 536
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

537

For peer review only

Figure 1

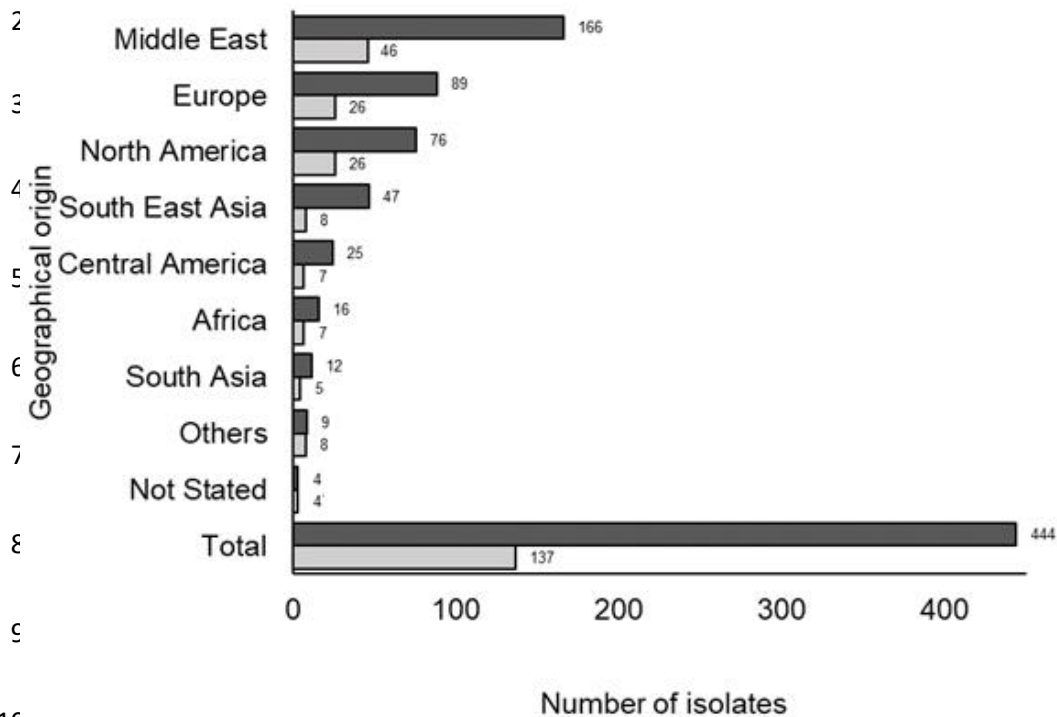
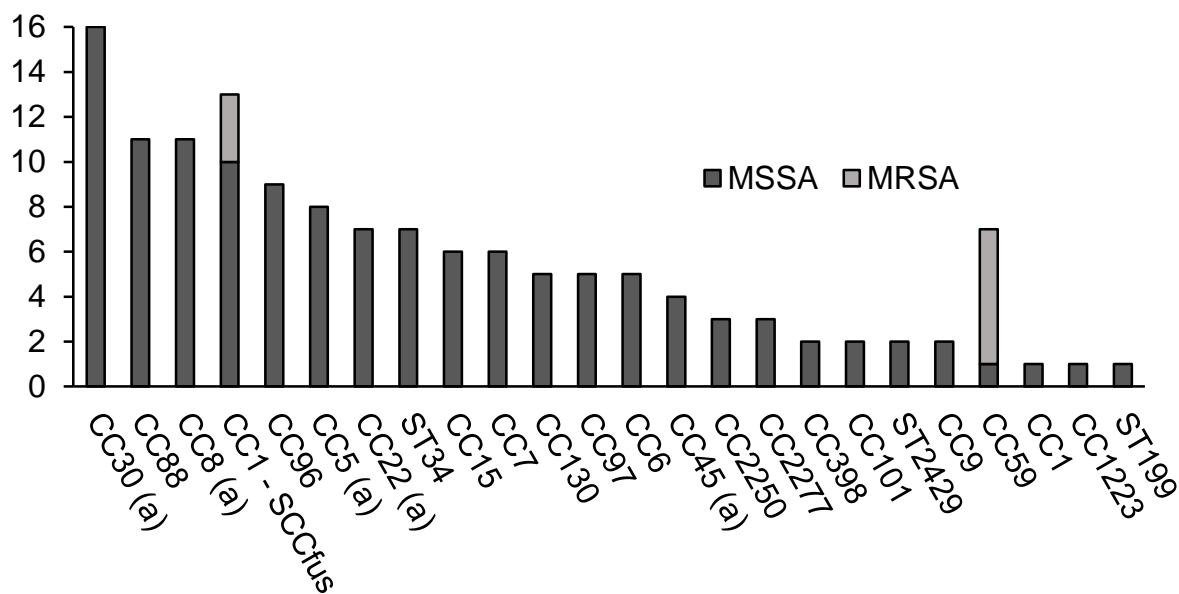


Figure 1. **Geographical origin of medical students recruited.** The geographical areas of origin of 444 medical students recruited to the study are shown (dark grey bars). Of those recruited, 137 were confirmed nasal *S. aureus* positive. The proportion of recruited students from each geographical origin with nasal *S. aureus* carriage are also shown (light grey bars).

1

Figure 2



2

3 Figure 1. Genotypic diversity of 137 *S. aureus* nasal isolates using DNA microarray
 4 analysis, including 128 MSSA (grey bars) and 9 MRSA (white bars). Bold lettering
 5 indicates internationally disseminated clones into which SCCmec can integrate.
 6 CC=clonal complex.

7

8

9

BMJ Open

An observational cross-sectional study of nasal staphylococcal species of medical students of diverse geographical origin, prior to healthcare exposure. Prevalence of SCCmec, fusC, fusB and the arginine catabolite mobile element (ACME) in the absence of selective antibiotic pressure.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020391.R1
Article Type:	Research
Date Submitted by the Author:	15-Jan-2018
Complete List of Authors:	Budri, Paulo; Royal College of Surgeons in Ireland, Clinical Microbiology Shore, Anna; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Coleman, David; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Kinnevey, Peter; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Humpreys, Hilary; Royal College of Surgeons in Ireland, Clinical Microbiology; Beaumont Hospital, Microbiology Department, Fitzgerald-Hughes, Deirdre; Royal College of Surgeons in Ireland, Clinical Microbiology
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Epidemiology
Keywords:	MRSA, antimicrobial resistance, nasal colonization, Coagulase negative staphylococci, <i>Staphylococcus aureus</i>

SCHOLARONE™
Manuscripts

1
2
3 1 **An observational cross-sectional study of nasal staphylococcal species of**
4 **medical students of diverse geographical origin, prior to healthcare exposure.**
5 2
6 3 **Prevalence of SCCmec, fusC, fusB and the arginine catabolite mobile element**
7
8 4 **(ACME) in the absence of selective antibiotic pressure.**
9

10
11 5 Paulo Eduardo Budri^{1*}, Anna C. Shore², David C. Coleman², Peter M. Kinnevey², Hilary
12
13 6 Humphreys^{1,3}, Deirdre Fitzgerald-Hughes¹.
14
15

16 7
17
18 8 ¹Department of Clinical Microbiology, Royal College of Surgeons in Ireland, Education
19 and Research Centre, Beaumont Hospital, Dublin 9, Ireland.

20 9
21
22 10 ²Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University
23 Hospital, University of Dublin, Trinity College, Dublin 2, Ireland
24
25

26 11
27 12 ³Microbiology Department, Beaumont Hospital, Dublin 9, Ireland
28
29

30 13
31
32 14 **Keywords;** *Staphylococcus aureus*, Coagulase-negative staphylococci, Nasal
33 colonization, Healthy human nares, Antimicrobial resistance, MRSA.
34
35

36 15
37 16 **Running title:** Antimicrobial resistance and virulence genes among staphylococcal
38 carriage isolates.
39
40

41 17
42 18
43
44 19 *Corresponding author: Paulo Eduardo Budri, Department of Clinical Microbiology,
45 RCSI Education and Research Centre, Smurfit Building, Beaumont Hospital, Dublin 9,
46 Ireland. Tel. +353 1 8093711, Fax +353 1 8092871, Email paulobudri@rcsi.com
47
48
49
50

1
2
3 24 **Abstract**
4

5 25 **Objective:** The aim of this study was to investigate co-located nasal *Staphylococcus*
6
7 26 *aureus* and coagulase-negative staphylococci (CoNS) (mainly *Staphylococcus*
8
9 27 *epidermidis*), recovered from healthy medical students in their pre-clinical year, prior to
10
11 28 exposure to the healthcare environment, for the carriage of genes and genetic
12
13 29 elements common to both species and that may contribute to *S. aureus* and methicillin-
14
15 30 resistant *S. aureus* (MRSA) evolution.

16
17 31 **Design:** Prospective observational cross-sectional study. Carriage of antimicrobial
18
19 32 resistance and virulence-associated genes in the absence of significant antibiotic
20
21 33 selective pressure was investigated among healthy medical students from
22
23 34 geographically diverse origins who were nasally co-colonised with *S. aureus* and
24
25 35 CoNS. Clonal lineages of *S. aureus* isolates were determined.

26
27 36 **Setting/Participants:** Dublin-based international undergraduate medical students
28

29 37 **Results:** Nasal *S. aureus* carriage was identified in 137/444 (30.8%) students of whom
30
31 38 nine (6.6%) carried MRSA (ST59-MRSA-IV (6/9), CC1-MRSA-V-SCC*fus* (3/9)). The
32
33 39 genes *mecA*, *fusB*, *ileS2*, *qacA/qacC* and the arginine catabolite mobile element
34
35 40 (ACME)-*arc* were detected among colonizing nasal staphylococci and had a
36
37 41 significantly greater association with CoNS than *S. aureus*. The rate of co-carriage of
38
39 42 any of these genes in *S. aureus*/CoNS pairs recovered from the same individual was
40
41 43 <1 %.

42
43 44 **Conclusions** The relatively high prevalence of these genes among CoNS of the
44
45 45 healthy human flora in the absence of significant antibiotic selective pressure is of
46
47 46 interest. Further research is required to determine what factors are involved and
48
49 47 whether these are modifiable to help prevent the emergence and spread of antibiotic
50
51 48 resistance amongst staphylococci.

52
53 49
54
55 50

1
2
3 514
5 52 **Strengths and limitations of this study:**

- 6
-
- 7 53 • Global evaluation of antibiotic resistance gene carriage among Staphylococci
-
- 8 54 among healthy medical students in preclinical years through DNA microarray
-
- 9 55 analyses.
-
- 10
-
- 11 56 • Pairs of staphylococcal species were isolated from the same colonisation site
-
- 12 57 (nares) of multiple participants to allow investigation of shared antibiotic
-
- 13 58 resistance and virulence in the same human niche in a community setting.
-
- 14
-
- 15 59 • A single centre study design.
-
- 16
-
- 17 60 • CoNS was investigated only in students co-colonised with
- S. aureus*
- .
-
- 18
-
- 19 61 • The study design did not facilitate follow-up of this cohort during clinical training.
-
- 20
-
- 21
-
- 22
-
- 23
-
- 24
-
- 25
-
- 26

27 63 **Introduction**28
29 64 *Staphylococcus aureus* and *Staphylococcus epidermidis* are significant colonisers of
30 65 healthy human skin and nares and are among the leading causes of healthcare-
31 66 associated infection (HAI). Morbidity, mortality and the financial burden associated with
32 67 methicillin-resistant *S. aureus* (MRSA) infections are well documented. Furthermore,
33 68 coagulase-negative staphylococci (CoNS) including *S. epidermidis* are reported
34 69 reservoirs of antimicrobial-resistance genes and their associated mobile genetic
35 70 elements, most notably the staphylococcal cassette chromosome (SCC) harbouring the
36 71 *mec* gene (SCC*mec*)¹.
37
38
39
40
41
42
43
44
4546 72 Twelve SCC*mec* types and numerous subtypes have been described among
47 73 MRSA isolates to date. The more prevalent and diverse range of SCCs and SCC*mec*
48 74 among CoNS further supports CoNS as a reservoir for antimicrobial resistance genes¹.
49
50 75 The identification of SCC, SCC*mec* and SCC-associated elements with other
51 76 antimicrobial and virulence genes and their epidemiological relationships among
52 77 clinical staphylococci has advanced our understanding of the role of CoNS in the
53
54
55
56
57
58
59
60

1
2
3 78 evolution of MRSA ². For example, the fusidic acid resistance gene *fusC* is associated
4
5 79 with SCC*mec* IV-SCC₄₇₆ and other SCC-like elements have been identified in *S.*
6
7 80 *aureus*, MRSA and CoNS and may contribute to MRSA emergence in countries with
8
9 81 significant fusidic acid usage. ³⁻⁵ Furthermore, the SCC-like arginine catabolic mobile
10
11 82 element (ACME) which enhances acid tolerance, is abundant among clinical CoNS
12
13 83 isolates, in particular *S. epidermidis* and *S. haemolyticus* ⁶. Among *S. aureus*, ACME
14
15 84 has mainly been detected among isolates of the community-associated (CA) USA300
16
17 85 clone ⁷. CoNS are also a putative reservoir of the high level mupirocin resistance
18
19 86 encoding gene *ileS2*, which is also increasing among *S. aureus*/MRSA in healthcare
20
21 87 and community environments related to horizontal gene transfer or expansion of
22
23 88 specific clones ^{8,9}.

24
25 89 Increasingly, MRSA clones previously associated with the community, such as clonal
26
27 90 complex (CC) 1 are spreading to healthcare settings making the differentiation between
28
29 91 healthcare-associated (HA) MRSA and CA-MRSA unclear ¹⁰. Therefore, detailed
30
31 92 investigation of the genetic and phenotypic traits of colonizing staphylococcal species
32
33 93 in community settings are important to identify those with features that may contribute
34
35 94 to their evolution into potentially successful and formidable healthcare-associated
36
37 95 clones. The aim of this study was to investigate co-located nasal *S. aureus* and CoNS
38
39 96 (mainly *S. epidermidis*) recovered from healthy medical students in their pre-clinical
40
41 97 year, prior to exposure to the healthcare environment, for the carriage of genes and
42
43 98 genetic elements common to both species and that may contribute to *S. aureus* and
44
45 99 MRSA evolution.

100 **Methods**

101 **Study setting, participants and sample collection**

102 This observational cross-sectional study was conducted at the Royal College of
103 Surgeons in Ireland (RCSI) from December 2014 – January 2016. Nasal swabs

1
2
3 104 (eSwab Copan®), Italy) were collected anonymously from undergraduate medical
4
5 105 students. Eligible students were those attending the RCSI medical centre to submit a
6
7 106 swab for mandatory MRSA screening in the week before they began their clinical
8
9 107 attachments. In total 444/450 eligible medical students (250 (56.3%) male, 194 (43.7%)
10
11 108 female) participated in this study. All participants reported no previous hospital contact
12
13 109 in the six weeks prior to recruitment. The student volunteers were from the second year
14
15 110 of the undergraduate medical programme and as such, all participants were domiciled
16
17 111 in Ireland for a minimum of two years prior to recruitment. Data was collected
18
19 112 anonymously from each participant, including age range, region of origin and previous
20
21 113 healthcare contact. Ethical approval (approval number REC949) was obtained from the
22
23 114 Institute's Ethics Committee and informed consent was obtained from each participant.

24 25 115 **Sample preparation**

26
27
28 116 Swabs were processed to recover *S. aureus* (including MRSA) and pathogenic CoNS
29
30 117 species using a modification of a published method¹¹. Swabs were enriched in brain
31
32 118 heart infusion (BHI) supplemented with 6% (w/v) NaCl for 24 h at 37°C followed by
33
34 119 further enrichment in mannitol salt broth for 24 h at 37°C. The enriched culture was
35
36 120 diluted 1/1000 and 100 µl was spread onto SaSelect agar (Bio-Rad®, Hercules, CA,
37
38 121 USA). Plates that yielded pink/orange colonies (presumptive *S. aureus*) were inspected
39
40 122 for growth of colonies of relevant CoNS based on colony colour (e.g. light pink colonies
41
42 123 of various sizes, presumptive *S. epidermidis*; white/yellow colonies, *S. haemolyticus*, *S.*
43
44 124 *hominis*, *S. capitis*, *S. warneri*, *S. caprae*, *S. lugdunensis*). Presumptive CoNS species
45
46 125 and *S. aureus* were sub-cultured from these plates onto Columbia blood agar (CBA)
47
48 126 and identified by matrix-assisted laser desorption/ionization-time-of-flight mass
49
50 127 spectrometry (MALDI-TOF) using a MALDI Biotyper (Microflex LT, Bruker). Matched
51
52 128 isolates (where *S. aureus* and a CoNS species were recovered from the same swab)
53
54 129 were cryopreserved and stored at -20°C (Protect™ bacterial preserver beads
55
56 130 (Technical Service Consultants, UK).

1
2
3 1314
5 132 **Characterisation of *S. aureus* and CoNS isolates**

6 133 Genomic DNA from *S. aureus* and CoNS isolates was extracted using enzymatic lysis
7 134 using the buffers and solutions provided with the *S. aureus* Genotyping Kit 2.0 (Alere
8 135 Technologies GmbH, Jena, Germany) and a DNeasy® Blood and Tissue kit (Qiagen,
9 136 Crawley, UK). Genetic characterisation of isolates was undertaken by DNA microarray
10 137 profiling using the *S. aureus* Genotyping Kit 2.0 as described previously^{12 13}. The kit
11 138 detects 333 gene targets including staphylococcal antimicrobial-resistance, virulence,
12 139 SCC*mec* and ACME-*arc* genes and assigns *S. aureus* isolates to multilocus sequence
13 140 type (ST) or clonal complexes (CC)s. MRSA phenotype was confirmed in *S. aureus*
14 141 and CoNS isolates positive for *mecA* by growth of pink (*S. aureus*) or colorless/white
15 142 (CoNS) colonies on MRSASelect agar (Bio-Rad®, Hercules, CA, USA). When required,
16 143 confirmation of carriage of fusidic acid resistance genes *fusC*, *fusB*, and toxic shock
17 144 syndrome toxin gene (*tst1*) were confirmed by PCR using the primers and conditions
18 145 described by O'Neill *et al*¹⁴ and Chen *et al*¹⁵.

19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35 14636
37 147 **Fusidic acid susceptibility testing**

38 148 Fusidic acid MICs were determined by ETEST® (bioMérieux, Marcy-l'Etoile, France)
39 149 according to manufacturer's instructions. Thirty *S. aureus* and CoNS isolates
40 150 harbouring *fusC* or *fusB* were sub-cultured twice before testing. Results were
41 151 interpreted according to EUCAST (<http://www.eucast.org>, assessed May 2015)
42 152 susceptibility criteria.

43
44
45
46
47
48 153 **Statistical analyses**

49 154 Fisher's exact test was used to analyze categorical variables (prevalence of genes)
50 155 using GraphPad QuickCalcs on-line software. The significance of differences between

156 groups was expressed as two-tailed p-values, *p* values of ≤ 0.05 were considered
157 statistically significant.

158

159 **Results**

160 **Nasal carriage of staphylococcal species and regional distribution**

161 Thirty-one percent (137/444) of students were positive for nasal carriage of *S. aureus*
162 of whom 6.6 % (9/137) were MRSA. Eighty-seven percent (386/444) of students were
163 positive for nasal carriage of CoNS (*S. epidermidis* (82% 364/444), *S. haemolyticus*
164 (3% 14/444) or *S. saprophyticus* (2% 8/444) (Table 1). All students positive for *S.*
165 *aureus* also carried *S. epidermidis*. Methicillin-resistant (MR)-CoNS were investigated
166 in the *S. aureus*-positive cohort only of which 13.1 % (18/137) were MR-CoNS. One
167 student exhibited co-carriage of MRSA and MR-CoNS. The geographical region of
168 origin of students harbouring *S. aureus* and CoNS is shown in Figure 1. The Middle
169 East, Europe and North America accounted for 68.6% of *S. aureus* carriers. For
170 regions represented by ≥ 12 participants, the rate of nasal carriage of *S. aureus* varied
171 geographically between 17 % (South East Asia) and 44 % (Africa).

172

173 **Clonal lineages among *S. aureus* isolates**

174 The ST or CC distribution among 137 *S. aureus* isolates is shown in Figure 2. Isolates
175 belonged to a variety of CCs with 46/137 (33.5%) assigned to internationally
176 disseminated CC5, CC8, CC22, CC30, CC45. A further 24/137 (17.5%) isolates
177 belonged to CC1, CC59, CC88 or CC398.

178

179 **SCCmec types and fusidic acid resistance among *S. aureus* and CoNS**

1
2
3 180 Of the 333 staphylococcal genes detected by the microarray, the two most prevalent
4
5 181 antibiotic resistance genes among nasal staphylococci were those encoding resistance
6
7 182 to β -lactams and fusidic acid. The most common SCC*mec* type among nasal MRSA
8
9 183 (n=9) and MR-CoNS (n=18) was SCC*mec* type IV (class B *mec* (*mecA*, *DmecR1*,
10
11 184 *ugpQ*) and *ccrA-2*, *ccrB-2*). The nine MRSA isolates belonged to ST59-MRSA-IV (6/9)
12
13 185 and CC1-MRSA-V-SCC*fus* (3/9) (class C *mec* (*mecA*, *ugpQ*) and *ccrC* and *fusC*
14
15 186 (Q6GD50) and *ccrA-1*, *ccrB-1*). Among 18 MR-CoNS identified (17 *S. epidermidis* and
16
17 187 1 *S. saprophyticus*), half harboured SCC*mec* type IV (8 *S. epidermidis* and the single
18
19 188 *S. saprophyticus*). SCC*mec* types II, V and VII were identified in three, five and one of
20
21 189 the remaining *S. epidermidis* isolates, respectively. Isolates from the one individual who
22
23 190 exhibited nasal co-carriage of MRSA and MR-CoNS (*S. epidermidis*) both harboured
24
25 191 SCC*mec* type IV (Table 2).

26
27 192 In addition to the three CC1-MRSA-V isolates that carried SCC*fus*, the fusidic acid
28
29 193 resistance genes *fusC* and *fusB* were identified in 28/128 (21.8%) and 2/128 (1.5%) of
30
31 194 methicillin-susceptible *S. aureus* (MSSA) isolates, respectively. Ten of the 28 *fusC*-
32
33 195 positive MSSA isolates belonged to CC1-MSSA-SCC*fus* and 18 were CC88-MSSA. All
34
35 196 10 CC1-MSSA-SCC*fus* isolates harboured a combination of SCC*fus* with the cassette
36
37 197 chromosome recombinase (*ccr*) genes, *ccrA-1* and *ccrB-1*. The two *fusB* positive
38
39 198 isolates belonged to CC5-MSSA and CC8-MSSA (Table 2). Among MR-CoNS, 27.7 %
40
41 199 (5/18) *S. epidermidis* isolates carried *fusC* (two of them also carried *ccr* genes *ccrA-1*
42
43 200 *ccrB-1*) and 50 % *fusB* (9/18, eight *S. epidermidis* and the one *S. saprophyticus*).
44
45 201 Among methicillin susceptible CoNS isolates, the *fusC* and *fusB* genes were identified
46
47 202 in 20/119 (16.8%, 18 *S. epidermidis* and two *S. saprophyticus*) and 18/119 (15.1%, all
48
49 203 *S. epidermidis*), respectively. One participant had nasal co-carriage of *fusC*-positive *S.*
50
51 204 *aureus* (CC88-MSSA) and CoNS (*S. epidermidis*).

52
53 205 All SCC*mec* positive staphylococci were confirmed to have an MRSA/MR-
54
55 206 CoNS phenotype. However, there was poor correlation between *fusC/fusB* carriage

1
2
3 207 and phenotypic fusidic acid resistance. Fusidic acid MICs for all *fusC* or *fusB*-positive
4 208 *S. aureus* and CoNS isolates are shown in Table 3. Phenotypic fusidic acid resistance
5
6 209 was confirmed (based on EUCAST breakpoints, MICs ≥ 1 $\mu\text{g/ml}$) in 23/32 (71.8%) *S.*
7
8 210 *aureus* and 20/38 (52.6%) CoNS nasal isolates harbouring either *fusC* or *fusB* (DNA
9
10 211 microarray result confirmed by PCR). Eight nasal isolates (three *S. aureus*, five *S.*
11
12 212 *epidermidis*) positive for *fusB* exhibited high level fusidic acid resistance (MIC \geq
13
14 213 32 $\mu\text{g/ml}$). Fusidic acid resistance was inducible in a further three *S. aureus* and seven
15
16 214 *S. epidermidis* isolates following incubation with 0.01 $\mu\text{g/ml}$ fusidic acid BHI agar.

215 **Other notable antimicrobial resistance genes among nasal *S. aureus* and CoNS**

216 Apart from SCC*mec* element and *fus* genes, other antimicrobial genes detected among
217 staphylococcal nasal flora were identified by DNA microarray. Tetracycline resistance
218 genes, *tet(K)* or *tet(M)*, were detected in 13/137 (9.5 %) of *S. aureus* isolates and 6/137
219 (4.3%) of the CoNS isolates. The quaternary ammonium compound resistance genes
220 (*qacA/qacC*), encoding antiseptic resistance, were significantly more prevalent among
221 CoNS isolates compared to *S. aureus* isolates (29/137 (21.2%) Vs 2/137 (1.4%),
222 $p < 0.0001$). Significantly more CoNS than *S. aureus* isolates carried *ileS2* encoding
223 high-level mupirocin resistance (11/137 (8%) vs 1/137 (0.72%), $p < 0.01$). However,
224 none of these genes were common to *S. aureus*/CoNS pairs recovered from the same
225 individual. The β -lactamase genes were abundant among *S. aureus* and CoNS; *blaZ*
226 was present in 101/137 (73.72%) *S. aureus* isolates and 92/137 (67.1%) CoNS isolate
227 and in 74/137 (54%) of individuals, these genes were common to *S. aureus*/CoNS
228 pairs from the same nares. A summary of the antibiotic resistance genes found among
229 *S. aureus* and CoNS is shown in Table 2. The staphylococcal isolates were negative
230 for all other antibiotic resistance genes spotted on the microarray.

231 **Virulence genes among nasal *S. aureus* and CoNS**

1
2
3 232 A single isolate, CC30-MSSA, was positive for the Panton-Valentine leucocidin genes
4 233 (*lukF/S-PV*). Among nasal staphylococci, ACME-*arc* was significantly associated with
5
6 234 CoNS compared to *S. aureus* (44/137 (32.1%) Vs 1/137 (0.7%)), $p < 0.0001$. The toxic
7
8 235 shock syndrome toxin gene *tst1* was identified in 33/137 (24.1%) nasal *S. aureus*
9
10 236 isolates. Unusually, DNA microarray identified *tst1* in two *S. epidermidis* isolates and
11
12 237 this was confirmed by PCR. ACME-*arc* was common to *S. aureus*/CoNS recovered
13
14 238 from the nares in one individual only. One hundred and two (74.4%) *S. aureus* isolates
15
16 239 encoded one or more enterotoxin genes. The enterotoxin gene cluster (*egc*), containing
17
18 240 *seg, sei, sem, sen, seo, seu*) was the most prevalent (48/102, 47%) followed by *seq/k*
19
20 241 (13/102, 12.7 %) and *sec/I* (7/102, 6.8 %). The staphylococcal isolates were negative
21
22 242 for all other toxin genes spotted on the microarray.
23
24
25
26
27

243

244 Discussion

245 Studies of staphylococcal carriage and epidemiology among the healthy
246 population in the absence of significant antibiotic pressure are important in identifying
247 the potential for pathogenic evolution. To our knowledge, this is the first study to co-
248 investigate CoNS and *S. aureus* when recovered together from the nares of healthy
249 pre-clinical medical students. The species distribution of nasal colonizing CoNS was
250 similar to other studies¹⁶ although the enrichment methods used here favoured *S.*
251 *aureus* and *S. epidermidis* and may explain the low prevalence of other CoNS species.
252 Our study revealed that, apart from the *bla* genes, which are abundant among
253 staphylococci, the rates of co-carriage of antibiotic resistance genes in paired *S.*
254 *aureus*/CoNS from the same individual were low in the community setting at <1%.
255 Rates of simultaneous carriage of antimicrobial resistance among nasal staphylococci
256 are likely to be higher under selective antibiotic pressure but few studies have
257 investigated this among patients. One small study of hospitalized patients with nasal
258 carriage of *S. aureus* and CoNS reported a rate of 12.5 % patients carrying MRSA and

1
2
3 259 MR-CoNS¹⁷. However, the authors reported only two cases where simultaneous
4
5 260 carriage of MR-CoNS and MRSA was detected and the strains involved carried
6
7 261 different SCC*mec* types. Despite negligible detection of co-species nasal carriage of
8
9 262 these genes in medical students prior to healthcare exposure, based on antimicrobial
10
11 263 resistance gene carriage by CoNS from this cohort, there is significant potential for
12
13 264 mobilisation of genes to *S. aureus* that may enhance its pathogenic potential in the
14
15 265 healthcare setting.

16
17 266 DNA microarray analyses revealed carriage of SCC*mec*, *fusC*, *fusB*, *ileS2*,
18
19 267 *qacA/qacC* and ACME-*arc* among colonising nasal staphylococci in individuals with no
20
21 268 previous healthcare exposure with greater prevalence among CoNS than *S. aureus*.
22
23 269 This pattern among pre-clinical medical students, supports CoNS as a reservoir with
24
25 270 potential to subsequently accelerate antimicrobial resistance and pathogenicity among
26
27 271 colonizing *S. aureus* in clinical environments under antibiotic selective pressure^{16 18 19}.

28
29
30 272 Despite considerable geographical distribution of the participants in this study, a
31
32 273 *S. aureus* nasal carriage rate in the community of 30.8% was recorded. In this study,
33
34 274 CC30, CC88 and CC8 were the most prevalent clones identified among nasal *S.*
35
36 275 *aureus*. CC30 is among the internationally disseminated clones in which SCC*mec* has
37
38 276 been acquired and is a successful colonising lineage, reported among HA and CA-
39
40 277 MRSA. Among medical students, these MSSA isolates may therefore represent a
41
42 278 significant pool for the uptake of SCC*mec* in a clinical setting. CC88 is frequently
43
44 279 isolated in Australia but in our study the geographical background of isolates was
45
46 280 mixed (including Middle East, Europe, South East Asia and Central America). CC8 is
47
48 281 associated with MRSA infection and is globally disseminated²⁰. Although CC30, CC88
49
50 282 and CC8 were prevalent among community MSSA isolates in this study, among the
51
52 283 relatively few MRSA recovered, none belonged to these CCs. Two CC/ST types
53
54 284 detected among MRSA recovered from healthy medical students in this study were
55
56 285 ST59-MRSA-IV and CC1-MRSA-V-SCC*fusC*. ST59 (Western Australian-MRSA-73) is

1
2
3 286 a sporadic Australian strain and apart from PVL-negativity, is indistinguishable from
4 287 USA1000 ²¹ In this study the geographical background of these isolates was wide
5
6 288 (Middle East, North America and South East Asia).
7

8
9 289 The identification of a significant reservoir of antibiotic resistance among
10 290 medical students prior to healthcare exposure in subsequent clinical years, highlights
11 291 the need for effective infection prevention and control policies in relation to hand
12 292 hygiene and surveillance. In the absence of antibiotic selective pressure, the colonising
13 293 MRSA rate appears relatively stable and in this study was 2 % (9/444), similar to rates
14 294 reported elsewhere ²². However, a previous study among medical interns in China
15 295 reported a nasal MRSA rate of 9.4% likely reflecting exposure to the healthcare
16 296 environment ²³. One study reported an increasing in carriage rates of MR-CoNS from
17 297 14% among medical student pre-internship, to 29.28% among interns ²⁴. Prevalence
18 298 rates of MR-CoNS in recent community-based surveys are variable but rates of 16.5%
19 299 ¹⁹ and 17.2% ²⁵ are reported in similar cohorts to this study where, of those colonised
20 300 with *S. aureus*, 13.1% carried MR-CoNS. SCCmec type IV, the smallest of the
21 301 SCCmec elements, was the most prevalent type among MRSA and MR-CoNS here
22 302 (66.6% and 50%). SCCmec IV has been detected in approximately 40% of methicillin-
23 303 resistant *S. epidermidis* identified in humans ²⁶. However, in this study SCCmec type V
24 304 was also represented among MRSA and MR-CoNS. While only one individual was
25 305 colonised with MRSA and MR-CoNS in this study (both SCCmec type IV), the
26 306 preponderance of SCCmec IV element in nasal MRSA and MR-CoNS suggests the
27 307 potential for *mecA* gene transfer among these species even in the absence of selective
28 308 pressure. The small size of this element, which has a low fitness cost, may enhance its
29 309 dissemination potential. ²⁷
30

31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51 310 Fusidic acid resistance among *S. aureus* from healthy carriers in nine European
52 311 countries in 2014 was reported to be <10 % ²⁸. However, we found a prevalence of
53 312 22.6% of *fusC/fusB* genes among healthy carriers. Fusidic acid resistance appears to
54
55
56
57

1
2
3 313 correlate with increased use of this agent. For example, in New Zealand, where it is
4
5 314 used as a first-line empiric agent for topical treatment of impetigo, prevalence rates of
6
7 315 resistance in community *S. aureus* isolates increased from 17% in 1999 to 29% in 2013
8
9 316 ²⁹. In Europe, fusidic acid is combined with β -lactams for the treatment of
10
11 317 staphylococcal bacteraemia, endocarditis, and osteomyelitis ³⁰ and is used widely in
12
13 318 the community for skin and soft tissue infections (SSTIs). A 2010 study of fusidic acid
14
15 319 resistance among *S. aureus* clinical isolates showed Greece and Ireland to have the
16
17 320 highest rates (52.5 and 19.9%) ³¹. *SCCfus* has been identified in the CC1 background
18
19 321 and more recently, in other lineages such as ST239 and ST779 ³²⁻³⁴. As highlighted
20
21 322 here in the absence of significant antibiotic pressure in the community, it appears that
22
23 323 this element is associated with MRSA and MSSA in the CC1 background. This genetic
24
25 324 platform, particularly when associated with *SCCmec* in a composite element (*SCCmec*
26
27 325 V+*SCCfus*) may enable the transfer of multi-drug resistance in a single transfer event.
28
29 326 The use of fusidic acid is un-regulated in some countries and hence it may be used
30
31 327 inappropriately in a community setting (for example in short or discontinuous doses).
32
33 328 Inappropriate use of fusidic acid may therefore favour co-selection of methicillin-
34
35 329 resistance among *S. aureus*. In addition, in this study, 14/18 (77%) of MR-CoNS were
36
37 330 positive for *fusC* or *fusB*. This association of resistances among the resident flora may
38
39 331 provide further opportunity for dissemination of MRSA driven by fusidic acid selective
40
41 332 pressure. Interestingly a positive correlation between carriage of *fusC/fusB* and
42
43 333 phenotypic resistance was observed in only 71.9 % and 53.6 % of *S. aureus* and CoNS
44
45 334 respectively. However, induction of gene expression with fusidic acid pre-incubation
46
47 335 gave better correlation (82.2% and 76.3 % correlation).

48
49 336 There were limitations to this study, which included; a single centred, relatively
50
51 337 small study. Some nasally abundant CoNS species, for example *Staphylococcus*
52
53 338 *lugdunensis* and *Staphylococcus hominis*, were under-represented as the enrichment
54
55 339 method favoured pathogenic staphylococci such as *S. aureus* and *S. epidermidis*.

1
2
3 340 CoNS was investigated only in those co-colonised with *S. aureus* and therefore
4
5 341 prevalence rates for genes among CoNS do not reflect the entire cohort. CCs and STs
6
7 342 were determined only among *S. aureus* as the high rate of genetic recombination
8
9 343 among CoNS makes strain typing unreliable. Although the microarray system used is
10
11 344 reported effective for staphylococcal species other than *S. aureus*³⁵, some gene
12
13 345 targets may be heterologous among staphylococci leading to false negatives. The
14
15 346 study design did not facilitate follow-up of this cohort during clinical training which may
16
17 347 have revealed further changes in gene carriage among colonising staphylococci.
18
19 348 However, the multi-national origin of the student body in our institution facilitated
20
21 349 analysis of a relatively broad geographic cohort in a single study and emphasises the
22
23 350 role that importation plays in *S. aureus* epidemiology. Unlike other studies of
24
25 351 staphylococci in the healthy human nares, pairs of staphylococcal species originating
26
27 352 from the same individual were investigated here for their resistance and virulence traits.
28
29 353 These data support a low rate of transfer of antibiotic resistance between colonising
30
31 354 staphylococcal species in the absence of healthcare contact. However, it is concerning
32
33 355 that similar *SCCmec* and *SCCfusC* types, in addition to *ileS2*, *qacA/qacB* and ACME
34
35 356 are carried among CoNS and *S. aureus* in healthy individuals who will have
36
37 357 subsequent roles in healthcare provision. Given the increasing emergence of HA-
38
39 358 MRSA with features of community strains, further mobilisation of these elements under
40
41 359 selective antibiotic pressure may enhance the transmission and success of *S. aureus*
42
43 360 in the healthcare environment.

361 **Funding**

46
47 362 PEB received funding for the study from Coordenação de Aperfeiçoamento de Pessoal
48
49 363 de Nível Superior (CAPES), Brazil under the Science without Borders Program. Grant
50
51 364 number 9172-13-0.

365 **Competing interests**

1
2
3 366 HH has received funding from Pfizer and Astellas outside the relevance of the
4
5 367 submitted work, all other authors report no competing interests.

6
7 368 **Contributor Statement**
8

9
10 369 PEB and DFH recruited students to the study, PEB conducted the laboratory work and
11
12 370 drafted the manuscript. DFH and HH conceived of the study and contributed to study
13
14 371 design. AS, PK and DC provided critical data interpretation and revised the drafted
15
16 372 work. All authors contributed to the final approved draft.
17

18 373 **Data sharing statement**
19

20
21 374 All data for these analyses are included in the manuscript. No additional data are
22
23 375 available.
24

25 376 **Acknowledgements**
26

27
28 377 We acknowledge the support of the Mercer's medical centre, RCSI and Ms Helen
29
30 378 Barry, Chief Medical Scientist, St. James' Hospital Dublin.
31

32
33 379
34

35 380
36
37

38 381 **Figure Legends**
39

40
41 382 **Figure 1. Geographical origin of medical students recruited.** The geographical
42
43 383 areas of origin of 444 medical students recruited to the study are shown (dark grey
44
45 384 bars). Of those recruited, 137 were confirmed nasal *S. aureus* and CoNS positive. The
46
47 385 proportion of recruited students from each geographical origin with nasal *S. aureus*
48
49 386 carriage are also shown (light grey bars).
50

51 387 **Figure 2.** Genotypic diversity of 137 *S. aureus* nasal isolates using DNA microarray
52
53 388 analysis, including 128 MSSA (dark grey bars) and 9 MRSA (light grey bars). Letter (a)
54
55
56
57
58
59
60

1
2
3 389 indicates internationally disseminated clones into which SCC*mec* can integrate.

4
5 390 CC=clonal complex.

6
7 391

8
9
10 392

11
12 393

13
14
15 394

16
17 395

18
19
20 396

21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

397 Table 1. Staphylococci species recovered from 444 nasal swabs.

398

Staphylococcal species recovered	Total n, % n=444	Methicillin- resistant phenotype n,% n=137 ^a	399 400
<i>S. aureus</i>	137, 30.8		401
MRSA		9, 6.6	
<i>S. epidermidis</i>	364, 81.9		402
MRSE		17, 12.4	
<i>S. haemolyticus</i>	14, 3.1		403
MRSH		0	404
<i>S. saprophyticus</i>	8, 1.8		
MRSS		1, 0.72	405
Co-carriage species			406
<i>S. aureus</i> + <i>S. epidermidis</i>	137, 30.8		407
MRSA + MR-CoNS		1, 0.72	
<i>fusC</i> positive <i>S. aureus</i> + CoNS		1, 0.72	408

^a CoNS were investigated only in those positive for nasal S.

1
2
3
4
5
6 409 *aureus* in the student cohort and not in all those recruited. MRSA= methicillin resistant *S. aureus*, MRSE + methicillin resistant *S. epidermidis*,
7
8 410 MRSH = methicillin resistant *S. haemolyticus*, MRSS = methicillin resistant *S. saprophyticus*
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

For peer review only

411 Table 2. Resistance/virulence genes detected among 137 co-located nasal *S. aureus*/CoNS pairs.

Detected Gene(s)	Phenotypic resistance/trait	No. isolates positive		<i>P</i> value	No. <i>S. aureus</i> /CoNS pairs positive (n)
		<i>S. aureus</i> n (%)	CoNS n (%)		
		n=137	n=137		
<i>Antibiotic resistance gene</i>					
<i>blaZ</i>	β-lactam	101 (73.7)	92 (67.1)	0.289	74
<i>fusB</i>	Fusidic acid	2 (1.5)	27 (19.7)	0.0002*	0
<i>fusC</i> ^a	Fusidic acid	31 (22.6)	25 (18.2)	0.159	1
<i>mecA</i>	Methicillin	9 (6.5)	18 (13.1)	0.103	1
<i>ileS2</i>	Mupirocin	1 (0.7)	11 (8.0)	0.005*	1
<i>qacA</i> and <i>qacC</i>	Quaternary ammonium salts	3 (2.2)	29 (21.2)	<0.0001*	0
<i>tet(K)</i> and <i>tet(M)</i>	Tetracycline	13 (9.5)	6 (4.4)	0.152	0
<i>erm(C)</i>	Macrolide/lincosamide	6 (4.3)	5 (3.6)	1.000	0
<i>msr(A)</i>	Macrolide	2 (1.45)	15 (10.9)	0.002*	1
<i>mph(C)</i>	Macrolide	0	15 (10.9)	<0.0001*	0
<i>dfpS1</i>	Trimethoprim	0	19 (13.8)	<0.0001*	0

	<i>vga</i>	Streptogramin A	1 (0.7)	6 (4.3)	0.120	0
	<i>Virulence</i>					
	ACME- <i>arc</i>	pH tolerance	1 (0.7)	44 (32.1)	<0.0001*	1
	<i>tst1^b</i>	Toxic shock toxin	33 (24.1)	2 (1.5)	<0.0001*	0

412 ^aassociated with SCC element, (*ccrA-1* and *ccrB-1*) in 13/137 *S. aureus*. ^b*tst1* confirmed by PCR. ACME = Arginine Catabolite Mobile Element. * indicates a
 413 statistically significant result by Fisher's exact test.

414

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

415

Table 3. Fusidic acid MICs for *S. aureus* and CoNS

	MIC ≤ 1 µg/ml	MIC ≥ 1 µg/ml	MIC ≥ 32 µg/ml
MIC Interpretation ^a	S	R	HR
	n (%)	n(%)	n (%)
<i>S. aureus</i> (n = 32)	9 (28.1)	20 (62.5)	3 (9.3)
CoNS (n = 38)	18 (47.4)	15 (39.5)	5 (13.2)

416

^aInterpretation based on The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017. <http://www.eucast.org>, S = Susceptible, R = Resistant, HR = high level resistant

417

418

419

420

421 **References**

422

- 423 1. Fluit AC, Carpaij N, Majoor EA, et al. Shared reservoir of *ccrB* gene sequences
424 between coagulase-negative staphylococci and methicillin-resistant
425 *Staphylococcus aureus*. *The Journal of antimicrobial chemotherapy*
426 2013;**68**(8):1707-13. doi: 10.1093/jac/dkt121 [published Online First:
427 2013/04/20]
- 428 2. Shore AC, Coleman DC. Staphylococcal cassette chromosome mec: recent
429 advances and new insights. *International journal of medical microbiology : IJMM*
430 2013;**303**(6-7):350-9. doi: 10.1016/j.ijmm.2013.02.002 [published Online First:
431 2013/03/19]
- 432 3. Hung WC, Chen HJ, Lin YT, et al. Skin Commensal Staphylococci May Act as
433 Reservoir for Fusidic Acid Resistance Genes. *PLoS One*
434 2015;10(11):e0143106. doi: 10.1371/journal.pone.0143106 [published Online
435 First: 2015/11/19]
- 436 4. Ellington MJ, Reuter S, Harris SR, et al. Emergent and evolving antimicrobial
437 resistance cassettes in community-associated fusidic acid and methicillin-
438 resistant *Staphylococcus aureus*. *International journal of antimicrobial agents*
439 2015;**45**(5):477-84. doi: 10.1016/j.ijantimicag.2015.01.009 [published Online
440 First: 2015/03/15]
- 441 5. Baines SL, Howden BP, Heffernan H, et al. Rapid Emergence and Evolution of
442 *Staphylococcus aureus* Clones Harboring *fusC*-Containing Staphylococcal
443 Cassette Chromosome Elements. *J Antimicrob Chemother* 2016;**60**(4):2359-65.
444 doi: 10.1128/aac.03020-15
- 445 6. Miragaia M, de Lencastre H, Perdreau-Remington F, et al. Genetic diversity of
446 arginine catabolic mobile element in *Staphylococcus epidermidis*. *PLoS One*

- 1
2
3 447 2009;**4**(11):e7722. doi: 10.1371/journal.pone.0007722 [published Online First:
4
5 448 2009/11/07]
- 6
7 449 7. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an
8
9 450 epidemic clone of community-acquired methicillin-resistant *Staphylococcus*
10
11 451 *aureus*. *Lancet* 2006;**367**(9512):731-9. doi: S0140-6736(06)68231-7 [pii]
12
13 452 10.1016/S0140-6736(06)68231-7 [doi] [published Online First: 2006/03/07]
- 14
15 453 8. Gonzalez-Dominguez M, Seral C, Potel C, et al. Genotypic and phenotypic
16
17 454 characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) clones
18
19 455 with high-level mupirocin resistance. *Diagn Microbiol Infect Dis* 2016;**85**(2):213-
20
21 456 7. doi: 10.1016/j.diagmicrobio.2016.02.021 [published Online First: 2016/05/03]
- 22
23 457 9. Bathoorn E, Hetem DJ, Alphenaar J, et al. Emergence of high-level mupirocin
24
25 458 resistance in coagulase-negative staphylococci associated with increased
26
27 459 short-term mupirocin use. *J Clin Microbiol* 2012;**50**(9):2947-50. doi:
28
29 460 10.1128/jcm.00302-12 [published Online First: 2012/07/05]
- 30
31 461 10. Earls MR, Kinnevey PM, Brennan GI, et al. The recent emergence in hospitals of
32
33 462 multidrug-resistant community-associated sequence type 1 and spa type t127
34
35 463 methicillin-resistant *Staphylococcus aureus* investigated by whole-genome
36
37 464 sequencing: Implications for screening. *PloS one* 2017;**12**(4):e0175542. doi:
38
39 465 10.1371/journal.pone.0175542
- 40
41 466 11. Huber H, Giezendanner N, Stephan R, et al. Genotypes, antibiotic resistance
42
43 467 profiles and microarray-based characterization of methicillin-resistant
44
45 468 *Staphylococcus aureus* strains isolated from livestock and veterinarians in
46
47 469 Switzerland. *Zoonoses and public health* 2011;**58**(5):343-9. doi: 10.1111/j.1863-
48
49 470 2378.2010.01353.x [published Online First: 2010/09/21]
- 50
51 471 12. Monecke S, Jatzwauk L, Weber S, et al. DNA microarray-based genotyping of
52
53 472 methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. *Clin*
54
55 473 *Microbiol Infect* 2008;**14**(6):534-45. doi: CLM1986 [pii]

- 1
2
3 474 10.1111/j.1469-0691.2008.01986.x [doi] [published Online First: 2008/04/01]
4
5 475 13. Monecke S, Slickers P, Ehricht R. Assignment of *Staphylococcus aureus* isolates to
6
7 476 clonal complexes based on microarray analysis and pattern recognition. *FEMS*
8
9 477 *Immunol Med Microbiol* 2008;**53**(2):237-51. doi: FIM426 [pii]
10
11 478 10.1111/j.1574-695X.2008.00426.x [doi] [published Online First: 2008/05/30]
12
13 479 14. O'Neill AJ, Larsen AR, Henriksen AS, et al. A fusidic acid-resistant epidemic strain
14
15 480 of *Staphylococcus aureus* carries the *fusB* determinant, whereas *fusA*
16
17 481 mutations are prevalent in other resistant isolates. *Antimicrobial agents and*
18
19 482 *chemotherapy* 2004;**48**(9):3594-7. doi: 10.1128/aac.48.9.3594-3597.2004
20
21 483 [published Online First: 2004/08/26]
22
23 484 15. Chen HJ, Hung WC, Tseng SP, et al. Fusidic acid resistance determinants in
24
25 485 *Staphylococcus aureus* clinical isolates. *Antimicrobial agents and*
26
27 486 *chemotherapy* 2010;**54**(12):4985-91. doi: 10.1128/aac.00523-10 [published
28
29 487 Online First: 2010/09/22]
30
31 488 16. Iravani Mohammad Abadi M, Moniri R, Khorshidi A, et al. Molecular Characteristics
32
33 489 of Nasal Carriage Methicillin-Resistant Coagulase Negative Staphylococci in
34
35 490 School Students. *Jundishapur journal of microbiology* 2015;**8**(6):e18591. doi:
36
37 491 10.5812/jjm.18591v2 [published Online First: 2015/08/25]
38
39 492 17. Faria NA, Conceicao T, Miragaia M, et al. Nasal carriage of methicillin resistant
40
41 493 staphylococci. *Microb Drug Resist* 2014;**20**(2):108-17. doi:
42
43 494 10.1089/mdr.2013.0197 [published Online First: 2014/02/26]
44
45 495 18. Jamaluddin TZ, Kuwahara-Arai K, Hisata K, et al. Extreme genetic diversity of
46
47 496 methicillin-resistant *Staphylococcus epidermidis* strains disseminated among
48
49 497 healthy Japanese children. *Journal of clinical microbiology* 2008;**46**(11):3778-
50
51 498 83. doi: 10.1128/jcm.02262-07 [published Online First: 2008/10/04]
52
53 499 19. Barbier F, Ruppe E, Hernandez D, et al. Methicillin-resistant coagulase-negative
54
55 500 staphylococci in the community: high homology of SCCmec IVa between
56
57
58
59
60

- 1
2
3 501 *Staphylococcus epidermidis* and major clones of methicillin-resistant
4 502 *Staphylococcus aureus*. *J Infect Dis* 2010;**202**(2):270-81. doi: 10.1086/653483
5 503 [doi] [published Online First: 2010/06/17]
6
7
8 504 20. Jimenez JN, Ocampo AM, Vanegas JM, et al. CC8 MRSA strains harboring
9 505 SCCmec type IVc are predominant in Colombian hospitals. *PloS one*
10 506 2012;**7**(6):e38576. doi: 10.1371/journal.pone.0038576 [published Online First:
11 507 2012/06/30]
12
13
14 508 21. Monecke S, Coombs G, Shore AC, et al. A field guide to pandemic, epidemic and
15 509 sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PloS one*
16 510 2011;**6**(4):e17936. doi: 10.1371/journal.pone.0017936 [published Online First:
17 511 2011/04/16]
18
19
20 512 22. Abroo S, Hosseini Jazani N, Sharifi Y. Methicillin-resistant *Staphylococcus aureus*
21 513 nasal carriage between healthy students of medical and nonmedical
22 514 universities. *American journal of infection control* 2017;**45**(7):709-12. doi:
23 515 10.1016/j.ajic.2017.02.034 [published Online First: 2017/04/01]
24
25
26 516 23. Ma XX, Sun DD, Wang S, et al. Nasal carriage of methicillin-resistant
27 517 *Staphylococcus aureus* among preclinical medical students: epidemiologic and
28 518 molecular characteristics of methicillin-resistant *S. aureus* clones. *Diagnostic*
29 519 *microbiology and infectious disease* 2011;**70**(1):22-30. doi:
30 520 10.1016/j.diagmicrobio.2010.12.004 [published Online First: 2011/04/26]
31
32
33 521 24. Baragundi MC, Solabannavar SS, Gokale SK, et al. Methicillin and multidrug
34 522 resistant coagulase negative staphylococcal nasal carriage in medical students.
35 523 *The Journal of communicable diseases* 2012;**44**(4):231-7. [published Online
36 524 First: 2012/12/01]
37
38
39 525 25. Du X, Zhu Y, Song Y, et al. Molecular analysis of *Staphylococcus epidermidis*
40 526 strains isolated from community and hospital environments in China. *PloS one*
41 527 2013;**8**(5):e62742. doi: 10.1371/journal.pone.0062742 [published Online First:
42 528 2013/05/16]
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 529 26. Miragaia M, Thomas JC, Couto I, et al. Inferring a population structure for
4
5 530 *Staphylococcus epidermidis* from multilocus sequence typing data. *Journal of*
6
7 531 *bacteriology* 2007;**189**(6):2540-52. doi: 10.1128/jb.01484-06 [published Online
8
9 532 First: 2007/01/16]
- 10
11 533 27. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant
12
13 534 *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA
14
15 535 strains? *Clinical infectious diseases: an official publication of the Infectious*
16
17 536 *Diseases Society of America* 2008;**46**(6):787-94. doi: 10.1086/528716
18
19 537 [published Online First: 2008/02/13]
- 20
21 538 28. den Heijer CD, van Bijnen EM, Paget WJ, et al. Fusidic acid resistance in
22
23 539 *Staphylococcus aureus* nasal carriage strains in nine European countries.
24
25 540 *Future Microbiol* 2014;**9**(6):737-45. doi: 10.2217/fmb.14.36 [published Online
26
27 541 First: 2014/07/22]
- 28
29 542 29. Williamson DA, Monecke S, Heffernan H, et al. High usage of topical fusidic acid
30
31 543 and rapid clonal expansion of fusidic acid-resistant *Staphylococcus aureus*: a
32
33 544 cautionary tale. *Clinical infectious diseases: an official publication of the*
34
35 545 *Infectious Diseases Society of America* 2014;**59**(10):1451-4. doi:
36
37 546 10.1093/cid/ciu658 [published Online First: 2014/08/21]
- 38
39 547 30. Whitby M. Fusidic acid in septicaemia and endocarditis. *International journal of*
40
41 548 *antimicrobial agents* 1999;**12**:S17-S22. doi: [http://dx.doi.org/10.1016/S0924-](http://dx.doi.org/10.1016/S0924-8579(98)00070-3)
42
43 549 [8579\(98\)00070-3](http://dx.doi.org/10.1016/S0924-8579(98)00070-3)
- 44
45 550 31. Castanheira M, Watters AA, Mendes RE, et al. Occurrence and molecular
46
47 551 characterization of fusidic acid resistance mechanisms among *Staphylococcus*
48
49 552 spp. from European countries (2008). *J Antimicrob Chemother*
50
51 553 2010;**65**(7):1353-8. doi: 10.1093/jac/dkq094 [published Online First:
52
53 554 2010/05/01]
- 54
55 555 32. Holden MT, Feil EJ, Lindsay JA, et al. Complete genomes of two clinical
56
57 556 *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and

- 1
2
3 557 drug resistance. *Proc Natl Acad Sci U S A* 2004;**101**(26):9786-91. doi:
4 558 10.1073/pnas.0402521101 [published Online First: 2004/06/24]
5
6 559 33. Kinnevey PM, Shore AC, Brennan GI, et al. Emergence of sequence type 779
7 560 methicillin-resistant *Staphylococcus aureus* harboring a novel pseudo
8 561 staphylococcal cassette chromosome mec (SCCmec)-SCC-SCCCRISPR
9 562 composite element in Irish hospitals. *Antimicrob Agents Chemother*
10 563 2013;**57**(1):524-31. doi: 10.1128/aac.01689-12 [published Online First:
11 564 2012/11/14]
12
13 565 34. Lin YT, Tsai JC, Chen HJ, et al. A novel staphylococcal cassette chromosomal
14 566 element, SCCfusC, carrying *fusC* and *speG* in fusidic acid-resistant methicillin-
15 567 resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*
16 568 2014;**58**(2):1224-7. doi: 10.1128/aac.01772-13 [published Online First:
17 569 2013/11/28]
18
19 570 35. Argudin MA, Vanderhaeghen W, Butaye P. Diversity of antimicrobial resistance and
20 571 virulence genes in methicillin-resistant non-*Staphylococcus aureus*
21 572 staphylococci from veal calves. *Research in veterinary science* 2015;**99**:10-6.
22 573 doi: 10.1016/j.rvsc.2015.01.004 [published Online First: 2015/02/01]
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37 574
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

575

576

For peer review only

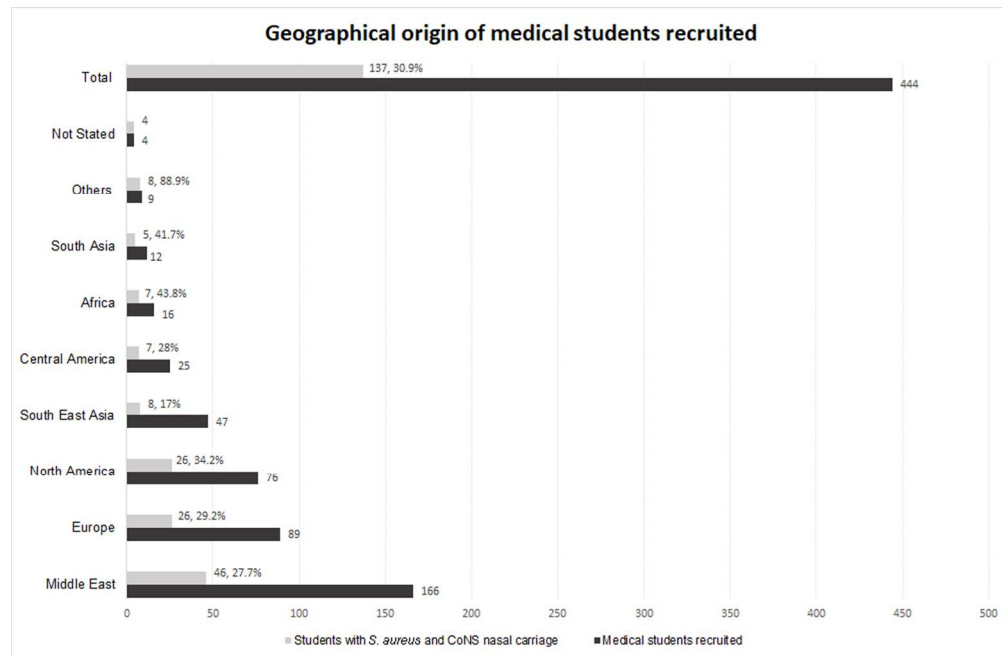


Figure 1. Geographical origin of medical students recruited. The geographical areas of origin of 444 medical students recruited to the study are shown (dark grey bars). Of those recruited, 137 were confirmed nasal *S. aureus* and CoNS positive. The proportion of recruited students from each geographical origin with nasal *S. aureus* carriage are also shown (light grey bars).

147x95mm (300 x 300 DPI)

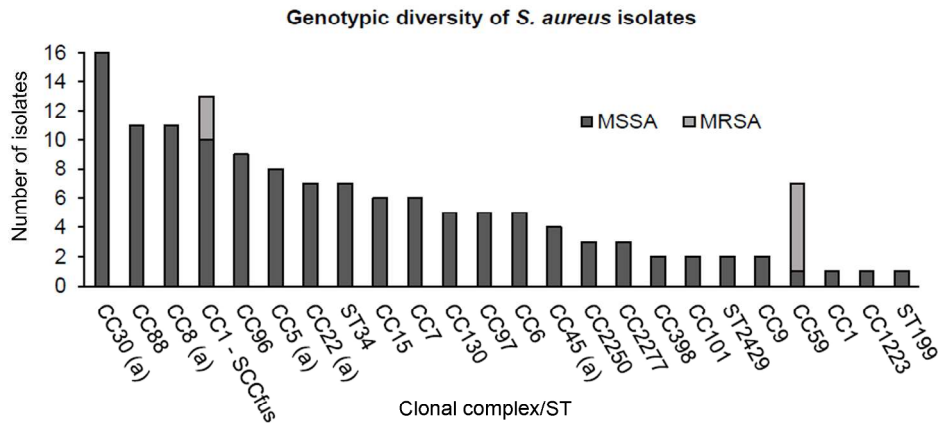


Figure 2. Genotypic diversity of 137 *S. aureus* nasal isolates using DNA microarray analysis, including 128 MSSA (dark grey bars) and 9 MRSA (light grey bars). Letter (a) indicates internationally disseminated clones into which SCC*mec* can integrate. CC=clonal complex.

222x127mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Relevant lines in manuscript and confirmation of items
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1- Title 1-Abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Yes
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Yes
Objectives	3	State specific objectives, including any prespecified hypotheses	25-29 (Abstract) 93-97 (Introduction)
Methods			
Study design	4	Present key elements of study design early in the paper	100
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	99-113
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	103-106
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	n/a n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	n/a
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	134-138 (Identification of genes) 146-149 (fusidic acid susceptibility)

			110-111 (participant details, age range, region of origin, healthcare exposure)
Bias	9	Describe any efforts to address potential sources of bias	102-106 (anonymous data collection, high participation rate no healthcare contact reported etc)
Study size	10	Explain how the study size was arrived at	105
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	152-154 (statistical analyses of variables)
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	152-154
		(b) Describe any methods used to examine subgroups and interactions	n/a
		(c) Explain how missing data were addressed	n/a
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	n/a
		(e) <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	n/a
		(e) Describe any sensitivity analyses	

Continued on next page

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60**Results**

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	105, 158
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	164-165 (relevant demographic region of origin)
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	n/a
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	n/a
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	n/a
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	158, 159 <i>S. aureus</i> and MRSA colonisation 162-163 MRSE among <i>S. aureus</i> colonised 163-163 Co-carriage of MRSA MRSE
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	179-183 SCCmec types among MRSA 183-186 SCCmec types among CoNS 189-194 fusC carriage among <i>S. aureus</i> and CoNS 200-201 Co-carriage of fus C 205-209 fusidic acid resistance among <i>S. aureus</i> /CoNS Confounders – n/a
		(b) Report category boundaries when continuous variables were categorized	n/a
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	249-254 257-260 285-288 300-303
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both	330-341

		direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	YES
Generalisability	21	Discuss the generalisability (external validity) of the study results	285-290 – comparison to other studies, stability of findings in relations to carriage rates
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	356-358

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

An observational cross-sectional study of nasal staphylococcal species of medical students of diverse geographical origin, prior to healthcare exposure. Prevalence of *SCCmec*, *fusC*, *fusB* and the arginine catabolite mobile element (ACME) in the absence of selective antibiotic pressure.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020391.R2
Article Type:	Research
Date Submitted by the Author:	23-Feb-2018
Complete List of Authors:	Budri, Paulo; Royal College of Surgeons in Ireland, Clinical Microbiology Shore, Anna; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Coleman, David; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Kinnevey, Peter; Dublin Dental University Hospital, University of Dublin, Trinity College, Microbiology Research Unit, Division of Oral Biosciences Humpreys, Hilary; Royal College of Surgeons in Ireland, Clinical Microbiology; Beaumont Hospital, Microbiology Department, Fitzgerald-Hughes, Deirdre; Royal College of Surgeons in Ireland, Clinical Microbiology
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Infectious diseases, Epidemiology
Keywords:	MRSA, antimicrobial resistance, nasal colonization, Coagulase negative staphylococci, <i>Staphylococcus aureus</i>

SCHOLARONE™
Manuscripts

1
2
3 1 **An observational cross-sectional study of nasal staphylococcal species of**
4 2 **medical students of diverse geographical origin, prior to healthcare exposure.**
5 3 **Prevalence of SCCmec, fusC, fusB and the arginine catabolite mobile element**
6 4 **(ACME) in the absence of selective antibiotic pressure.**
7
8
9

10
11 5 Paulo Eduardo Budri^{1*}, Anna C. Shore², David C. Coleman², Peter M. Kinnevey², Hilary
12 6 Humphreys^{1,3}, Deirdre Fitzgerald-Hughes¹.
13
14
15

16 7
17
18 8 ¹Department of Clinical Microbiology, Royal College of Surgeons in Ireland, Education
19 9 and Research Centre, Beaumont Hospital, Dublin 9, Ireland.

20 10 ²Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University
21 11 Hospital, University of Dublin, Trinity College, Dublin 2, Ireland

22 12 ³Microbiology Department, Beaumont Hospital, Dublin 9, Ireland
23
24
25
26
27
28
29
30
31
32

33 14 **Keywords;** *Staphylococcus aureus*, Coagulase-negative staphylococci, Nasal
34 15 colonization, Healthy human nares, Antimicrobial resistance, MRSA.

35 16 **Running title:** Antimicrobial resistance and virulence genes among staphylococcal
36 17 carriage isolates.
37
38
39
40
41
42
43

44 19 *Corresponding author: Paulo Eduardo Budri, Department of Clinical Microbiology,
45 20 RCSI Education and Research Centre, Smurfit Building, Beaumont Hospital, Dublin 9,
46 21 Ireland. Tel. +353 1 8093711, Fax +353 1 8092871, Email paulobudri@rcsi.com
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 24 **Abstract**
4

5 25 **Objective:** The aim of this study was to investigate co-located nasal *Staphylococcus*
6
7 26 *aureus* and coagulase-negative staphylococci (CoNS) (mainly *Staphylococcus*
8
9 27 *epidermidis*), recovered from healthy medical students in their pre-clinical year, prior to
10
11 28 exposure to the healthcare environment, for the carriage of genes and genetic
12
13 29 elements common to both species and that may contribute to *S. aureus* and methicillin-
14
15 30 resistant *S. aureus* (MRSA) evolution.

16
17 31 **Design:** Prospective observational cross-sectional study. Carriage of antimicrobial
18
19 32 resistance and virulence-associated genes in the absence of significant antibiotic
20
21 33 selective pressure was investigated among healthy medical students from
22
23 34 geographically diverse origins who were nasally co-colonised with *S. aureus* and
24
25 35 CoNS. Clonal lineages of *S. aureus* isolates were determined.

26
27 36 **Setting/Participants:** Dublin-based international undergraduate medical students
28

29 37 **Results:** Nasal *S. aureus* carriage was identified in 137/444 (30.8%) students of whom
30
31 38 nine (6.6%) carried MRSA (ST59-MRSA-IV (6/9), CC1-MRSA-V-SCC*fus* (3/9)). The
32
33 39 genes *mecA*, *fusB*, *ileS2*, *qacA/qacC* and the arginine catabolite mobile element
34
35 40 (ACME)-*arc* were detected among colonizing nasal staphylococci and had a
36
37 41 significantly greater association with CoNS than *S. aureus*. The rate of co-carriage of
38
39 42 any of these genes in *S. aureus*/CoNS pairs recovered from the same individual was
40
41 43 <1 %.

42
43 44 **Conclusions** The relatively high prevalence of these genes among CoNS of the
44
45 45 healthy human flora in the absence of significant antibiotic selective pressure is of
46
47 46 interest. Further research is required to determine what factors are involved and
48
49 47 whether these are modifiable to help prevent the emergence and spread of antibiotic
50
51 48 resistance amongst staphylococci.

52
53 49
54
55 50

1
2
3 514
5 52 **Strengths and limitations of this study:**

- 6
-
- 7 53 • Global evaluation of antibiotic resistance gene carriage among Staphylococci
-
- 8 54 among healthy medical students in preclinical years through DNA microarray
-
- 9 55 analyses.
-
- 10
-
- 11 56 • Pairs of staphylococcal species were isolated from the same colonisation site
-
- 12 57 (nares) of multiple participants to allow investigation of shared antibiotic
-
- 13 58 resistance and virulence in the same human niche in a community setting.
-
- 14
-
- 15 59 • A single centre study design.
-
- 16
-
- 17 60 • CoNS was investigated only in students co-colonised with
- S. aureus*
- .
-
- 18
-
- 19 61 • The study design did not facilitate follow-up of this cohort during clinical training.
-
- 20
-
- 21
-
- 22
-
- 23
-
- 24
-
- 25
-
- 26

27 63 **Introduction**28
29 64 *Staphylococcus aureus* and *Staphylococcus epidermidis* are significant colonisers of
30 65 healthy human skin and nares and are among the leading causes of healthcare-
31 66 associated infection (HAI). Morbidity, mortality and the financial burden associated with
32 67 methicillin-resistant *S. aureus* (MRSA) infections are well documented. Furthermore,
33 68 coagulase-negative staphylococci (CoNS) including *S. epidermidis* are reported
34 69 reservoirs of antimicrobial-resistance genes and their associated mobile genetic
35 70 elements, most notably the staphylococcal cassette chromosome (SCC) harbouring the
36 71 *mec* gene (SCC*mec*)¹.
37
38
39
40
41
42
43
44
4546 72 Twelve SCC*mec* types and numerous subtypes have been described among
47 73 MRSA isolates to date. The more prevalent and diverse range of SCCs and SCC*mec*
48 74 among CoNS further supports CoNS as a reservoir for antimicrobial resistance genes¹.
49
50 75 The identification of SCC, SCC*mec* and SCC-associated elements with other
51 76 antimicrobial and virulence genes and their epidemiological relationships among
52 77 clinical staphylococci has advanced our understanding of the role of CoNS in the
53
54
55
56
57
58
59
60

1
2
3 78 evolution of MRSA ². For example, the fusidic acid resistance gene *fusC* is associated
4
5 79 with SCC*mec* IV-SCC₄₇₆ and other SCC-like elements have been identified in *S.*
6
7 80 *aureus*, MRSA and CoNS and may contribute to MRSA emergence in countries with
8
9 81 significant fusidic acid usage. ³⁻⁵ Furthermore, the SCC-like arginine catabolic mobile
10
11 82 element (ACME) which enhances acid tolerance, is abundant among clinical CoNS
12
13 83 isolates, in particular *S. epidermidis* and *S. haemolyticus* ⁶. Among *S. aureus*, ACME
14
15 84 has mainly been detected among isolates of the community-associated (CA) USA300
16
17 85 clone ⁷. CoNS are also a putative reservoir of the high level mupirocin resistance
18
19 86 encoding gene *ileS2*, which is also increasing among *S. aureus*/MRSA in healthcare
20
21 87 and community environments related to horizontal gene transfer or expansion of
22
23 88 specific clones ^{8,9}.

24
25 89 Increasingly, MRSA clones previously associated with the community, such as clonal
26
27 90 complex (CC) 1 are spreading to healthcare settings making the differentiation between
28
29 91 healthcare-associated (HA) MRSA and CA-MRSA unclear ¹⁰. Therefore, detailed
30
31 92 investigation of the genetic and phenotypic traits of colonizing staphylococcal species
32
33 93 in community settings are important to identify those with features that may contribute
34
35 94 to their evolution into potentially successful and formidable healthcare-associated
36
37 95 clones. The aim of this study was to investigate co-located nasal *S. aureus* and CoNS
38
39 96 (mainly *S. epidermidis*) recovered from healthy medical students in their pre-clinical
40
41 97 year, prior to exposure to the healthcare environment, for the carriage of genes and
42
43 98 genetic elements common to both species and that may contribute to *S. aureus* and
44
45 99 MRSA evolution.

100 **Methods**

101 **Study setting, participants and sample collection**

102 This observational cross-sectional study was conducted at the Royal College of
103 Surgeons in Ireland (RCSI) from December 2014 – January 2016. Nasal swabs

1
2
3 104 (eSwab Copan®), Italy) were collected anonymously from undergraduate medical
4
5 105 students. Eligible students were those attending the RCSI medical centre to submit a
6
7 106 swab for mandatory MRSA screening in the week before they began their clinical
8
9 107 attachments. In total 444/450 eligible medical students (250 (56.3%) male, 194 (43.7%)
10
11 108 female) participated in this study. All participants reported no previous hospital contact
12
13 109 in the six weeks prior to recruitment. The student volunteers were from the second year
14
15 110 of the undergraduate medical programme and as such, all participants were domiciled
16
17 111 in Ireland for a minimum of two years prior to recruitment. Data was collected
18
19 112 anonymously from each participant, including age range, region of origin and previous
20
21 113 healthcare contact. Ethical approval (approval number REC949) was obtained from the
22
23 114 Institute's Ethics Committee and informed consent was obtained from each participant.
24
25
26
27

28 116 **Sample preparation**

29
30 117 Swabs were processed to recover *S. aureus* (including MRSA) and pathogenic CoNS
31
32 118 species using a modification of a published method¹¹. Swabs were enriched in brain
33
34 119 heart infusion (BHI) supplemented with 6% (w/v) NaCl for 24 h at 37°C followed by
35
36 120 further enrichment in mannitol salt broth for 24 h at 37°C. The enriched culture was
37
38 121 diluted 1/1000 and 100 µl was spread onto SaSelect agar (Bio-Rad®, Hercules, CA,
39
40 122 USA). Plates that yielded pink/orange colonies (presumptive *S. aureus*) were inspected
41
42 123 for growth of colonies of relevant CoNS based on colony colour (e.g. light pink colonies
43
44 124 of various sizes, presumptive *S. epidermidis*; white/yellow colonies, *S. haemolyticus*, *S.*
45
46 125 *hominis*, *S. capitis*, *S. warneri*, *S. caprae*, *S. lugdunensis*). Presumptive CoNS species
47
48 126 and *S. aureus* were sub-cultured from these plates onto Columbia blood agar (CBA)
49
50 127 and identified by matrix-assisted laser desorption/ionization-time-of-flight mass
51
52 128 spectrometry (MALDI-TOF) using a MALDI Biotyper (Microflex LT, Bruker). Matched
53
54 129 isolates (where *S. aureus* and a CoNS species were recovered from the same swab)
55
56
57
58
59
60

1
2
3 130 were cryopreserved and stored at -20°C (Protect™ bacterial preserver beads
4 131 (Technical Service Consultants, UK).

5
6
7 132

8 9 133 **Characterisation of *S. aureus* and CoNS isolates**

10
11 134 Genomic DNA from *S. aureus* and CoNS isolates was extracted using enzymatic lysis
12
13 135 using the buffers and solutions provided with the *S. aureus* Genotyping Kit 2.0 (Alere
14
15 136 Technologies GmbH, Jena, Germany) and a DNeasy® Blood and Tissue kit (Qiagen,
16
17 137 Crawley, UK). Genetic characterisation of isolates was undertaken by DNA microarray
18
19 138 profiling using the *S. aureus* Genotyping Kit 2.0 as described previously^{12 13}. The kit
20
21 139 detects 333 gene targets including staphylococcal antimicrobial-resistance, virulence,
22
23 140 SCC*mec* and ACME-*arc* genes and assigns *S. aureus* isolates to multilocus sequence
24
25 141 type (ST) or clonal complexes (CC)s. MRSA phenotype was confirmed in *S. aureus*
26
27 142 and CoNS isolates positive for *mecA* by growth of pink (*S. aureus*) or colorless/white
28
29 143 (CoNS) colonies on MRSASelect agar (Bio-Rad®, Hercules, CA, USA). When required,
30
31 144 confirmation of carriage of fusidic acid resistance genes *fusC*, *fusB*, and toxic shock
32
33 145 syndrome toxin gene (*tst1*) were confirmed by PCR using the primers and conditions
34
35 146 described by O'Neill *et al*¹⁴ and Chen *et al*¹⁵.

36
37 147

38 39 148 **Fusidic acid susceptibility testing**

40
41
42 149 Fusidic acid MICs were determined by ETEST® (bioMérieux, Marcy-l'Etoile, France)
43
44 150 according to manufacturer's instructions. Thirty *S. aureus* and CoNS isolates
45
46 151 harbouring *fusC* or *fusB* were sub-cultured twice before testing. Results were
47
48 152 interpreted according to EUCAST (<http://www.eucast.org>, assessed May 2015)
49
50 153 susceptibility criteria.

51 52 53 154 **Statistical analyses**

1
2
3 155 Fisher's exact test was used to analyze categorical variables (prevalence of genes)
4
5 156 using GraphPad QuickCalcs on-line software. The significance of differences between
6
7 157 groups was expressed as two-tailed p-values, *p* values of ≤ 0.05 were considered
8
9 158 statistically significant.

10 159 **Patient and Public Involvement statement:**

11
12 160 The participants in this study were medical undergraduate students. They were invited
13
14 161 to participate in this study when they underwent mandatory MRSA screening prior to
15
16 162 commencement of clinical placements. As future clinicians, participants were broadly
17
18 163 considered in the development of the research question. As nasal carriage of *S. aureus*
19
20 164 and MRSA among healthcare staff contributes to transmission to patients in healthcare
21
22 165 environments, medical students have an interest in contributing to this knowledge.
23
24 166 Participants did not contribute to the study design. Participants were informed at
25
26 167 recruitment that dissemination of the results would be through the scientific literature.
27
28 168

29 30 31 169 **Results**

32 33 170 **Nasal carriage of staphylococcal species and regional distribution**

34
35
36 171 Thirty-one percent (137/444) of students were positive for nasal carriage of *S. aureus*
37
38 172 of whom 6.6 % (9/137) were MRSA. Eighty-seven percent (386/444) of students were
39
40 173 positive for nasal carriage of CoNS (*S. epidermidis* (82% 364/444), *S. haemolyticus*
41
42 174 (3% 14/444) or *S. saprophyticus* (2% 8/444) (Table 1). All students positive for *S.*
43
44 175 *aureus* also carried *S. epidermidis*. Methicillin-resistant (MR)-CoNS were investigated
45
46 176 in the *S. aureus*-positive cohort only of which 13.1 % (18/137) were MR-CoNS. One
47
48 177 student exhibited co-carriage of MRSA and MR-CoNS. The geographical region of
49
50 178 origin of students harbouring *S. aureus* and CoNS is shown in Figure 1. The Middle
51
52 179 East, Europe and North America accounted for 68.6% of *S. aureus* carriers. For
53
54 180 regions represented by ≥ 12 participants, the rate of nasal carriage of *S. aureus* varied
55
56 181 geographically between 17 % (South East Asia) and 44 % (Africa).

182

183 Clonal lineages among *S. aureus* isolates

184 The ST or CC distribution among 137 *S. aureus* isolates is shown in Figure 2. Isolates
185 belonged to a variety of CCs with 46/137 (33.5%) assigned to internationally
186 disseminated CC5, CC8, CC22, CC30, CC45. A further 24/137 (17.5%) isolates
187 belonged to CC1, CC59, CC88 or CC398.

188

189 SCCmec types and fusidic acid resistance among *S. aureus* and CoNS

190 Of the 333 staphylococcal genes detected by the microarray, the two most prevalent
191 antibiotic resistance genes among nasal staphylococci were those encoding resistance
192 to β -lactams and fusidic acid. The most common SCCmec type among nasal MRSA
193 (n=9) and MR-CoNS (n=18) was SCCmec type IV (class B *mec* (*mecA*, *DmecR1*,
194 *ugpQ*) and *ccrA-2*, *ccrB-2*). The nine MRSA isolates belonged to ST59-MRSA-IV (6/9)
195 and CC1-MRSA-V-SCCfus (3/9) (class C *mec* (*mecA*, *ugpQ*) and *ccrC* and *fusC*
196 (Q6GD50) and cassette chromosome recombinase (*ccr*) *A-1*, *ccrB-1*). Among 18 MR-
197 CoNS identified (17 *S. epidermidis* and 1 *S. saprophyticus*), half harboured SCCmec
198 type IV (8 *S. epidermidis* and the single *S. saprophyticus*). SCCmec types II, V and VII
199 were identified in three, five and one of the remaining *S. epidermidis* isolates,
200 respectively. Isolates from the one individual who exhibited nasal co-carriage of MRSA
201 and MR-CoNS (*S. epidermidis*) both harboured SCCmec type IV (Table 2).

202 In addition to the three CC1-MRSA-V isolates that carried SCCfus, the fusidic acid
203 resistance genes *fusC* and *fusB* were identified in 28/128 (21.8%) and 2/128 (1.5%) of
204 methicillin-susceptible *S. aureus* (MSSA) isolates, respectively. Ten of the 28 *fusC*-
205 positive MSSA isolates belonged to CC1-MSSA-SCCfus, 11 were CC88-MSSA and 7
206 CC8-MSSA. All 10 CC1-MSSA-SCCfus isolates harboured a combination of SCCfus
207 with the *ccr* genes, *ccrA-1* and *ccrB-1*. The two *fusB* positive isolates belonged to CC5-

1
2
3 208 MSSA and CC8-MSSA (Table 2). Among MR-CoNS, 27.7 % (5/18) *S. epidermidis*
4
5 209 isolates carried *fusC* (two of them also carried *ccr* genes *ccrA-1 ccrB-1*) and 50 % *fusB*
6
7 210 (9/18, eight *S. epidermidis* and the one *S. saprophyticus*). Among methicillin
8
9 211 susceptible CoNS isolates, the *fusC* and *fusB* genes were identified in 20/119 (16.8%,
10
11 212 18 *S. epidermidis* and two *S. saprophyticus*) and 18/119 (15.1%, all *S. epidermidis*),
12
13 213 respectively. One participant had nasal co-carriage of *fusC*-positive *S. aureus* (CC88-
14
15 214 MSSA) and CoNS (*S. epidermidis*).

16
17 215 All SCC*mec* positive staphylococci were confirmed to have an MRSA/MR-
18
19 216 CoNS phenotype. However, there was poor correlation between *fusC/fusB* carriage
20
21 217 and phenotypic fusidic acid resistance. Fusidic acid MICs for all *fusC* or *fusB*-positive
22
23 218 *S. aureus* and CoNS isolates are shown in Table 3. Phenotypic fusidic acid resistance
24
25 219 was confirmed (based on EUCAST breakpoints, MICs ≥ 1 $\mu\text{g/ml}$) in 23/32 (71.8%) *S.*
26
27 220 *aureus* and 20/38 (52.6%) CoNS nasal isolates harbouring either *fusC* or *fusB* (DNA
28
29 221 microarray result confirmed by PCR). Eight nasal isolates (three *S. aureus*, five *S.*
30
31 222 *epidermidis*) positive for *fusB* exhibited high level fusidic acid resistance (MIC \geq
32
33 223 32 $\mu\text{g/ml}$). Fusidic acid resistance was inducible in a further three *S. aureus* and seven
34
35 224 *S. epidermidis* isolates following incubation with 0.01 $\mu\text{g/ml}$ fusidic acid BHI agar.

36 37 38 225 **Other notable antimicrobial resistance genes among nasal *S. aureus* and CoNS**

39
40 226 Apart from SCC*mec* element and *fus* genes, other antimicrobial genes detected among
41
42 227 staphylococcal nasal flora were identified by DNA microarray. Tetracycline resistance
43
44 228 genes, *tet(K)* or *tet(M)*, were detected in 13/137 (9.5 %) of *S. aureus* isolates and 6/137
45
46 229 (4.3%) of the CoNS isolates. The quaternary ammonium compound resistance genes
47
48 230 (*qacA/qacC*), encoding antiseptic resistance, were significantly more prevalent among
49
50 231 CoNS isolates compared to *S. aureus* isolates (29/137 (21.2%) Vs 2/137 (1.4%),
51
52 232 $p < 0.0001$). Significantly more CoNS than *S. aureus* isolates carried *ileS2* encoding
53
54 233 high-level mupirocin resistance (11/137 (8%) vs 1/137 (0.72%), $p < 0.01$). However,
55
56 234 none of these genes were common to *S. aureus*/CoNS pairs recovered from the same

individual. The β -lactamase genes were abundant among *S. aureus* and CoNS; *blaZ* was present in 101/137 (73.72%) *S. aureus* isolates and 92/137 (67.1%) CoNS isolate and in 74/137 (54%) of individuals, these genes were common to *S. aureus*/CoNS pairs from the same nares. A summary of the antibiotic resistance genes found among *S. aureus* and CoNS is shown in Table 2. The staphylococcal isolates were negative for all other antibiotic resistance genes spotted on the microarray.

241 **Virulence genes among nasal *S. aureus* and CoNS**

A single isolate, CC30-MSSA, was positive for the Panton-Valentine leucocidin genes (*lukF/S-PV*). Among nasal staphylococci, ACME-*arc* was significantly associated with CoNS compared to *S. aureus* (44/137 (32.1%) Vs 1/137 (0.7%)), $p < 0.0001$. The toxic shock syndrome toxin gene *tst1* was identified in 33/137 (24.1%) nasal *S. aureus* isolates. Unusually, DNA microarray identified *tst1* in two *S. epidermidis* isolates and this was confirmed by PCR. ACME-*arc* was common to *S. aureus*/CoNS recovered from the nares in one individual only. One hundred and two (74.4%) *S. aureus* isolates encoded one or more enterotoxin genes. The enterotoxin gene cluster (*egc*), containing *seg*, *sei*, *sem*, *sen*, *seo*, *seu*) was the most prevalent (48/102, 47%) followed by *seq/k* (13/102, 12.7 %) and *sec/l* (7/102, 6.8 %). The staphylococcal isolates were negative for all other toxin genes spotted on the microarray.

253

254 **Discussion**

255 Studies of staphylococcal carriage and epidemiology among the healthy
256 population in the absence of significant antibiotic pressure are important in identifying
257 the potential for pathogenic evolution. To our knowledge, this is the first study to co-
258 investigate CoNS and *S. aureus* when recovered together from the nares of healthy
259 pre-clinical medical students. The species distribution of nasal colonizing CoNS was
260 similar to other studies¹⁶ although the enrichment methods used here favoured *S.*

1
2
3 261 *aureus* and *S. epidermidis* and may explain the low prevalence of other CoNS species.
4
5 262 Our study revealed that, apart from the *bla* genes, which are abundant among
6
7 263 staphylococci, the rates of co-carriage of antibiotic resistance genes in paired *S.*
8
9 264 *aureus*/CoNS from the same individual were low in the community setting at <1%.
10
11 265 Rates of simultaneous carriage of antimicrobial resistance among nasal staphylococci
12
13 266 are likely to be higher under selective antibiotic pressure but few studies have
14
15 267 investigated this among patients. One small study of hospitalized patients with nasal
16
17 268 carriage of *S. aureus* and CoNS reported a rate of 12.5 % patients carrying MRSA and
18
19 269 MR-CoNS¹⁷. However, the authors reported only two cases where simultaneous
20
21 270 carriage of MR-CoNS and MRSA was detected and the strains involved carried
22
23 271 different *SCCmec* types. Despite negligible detection of co-species nasal carriage of
24
25 272 these genes in medical students prior to healthcare exposure, based on antimicrobial
26
27 273 resistance gene carriage by CoNS from this cohort, there is significant potential for
28
29 274 mobilisation of genes to *S. aureus* that may enhance its pathogenic potential in the
30
31 275 healthcare setting.

32
33 276 DNA microarray analyses revealed carriage of *SCCmec*, *fusC*, *fusB*, *ileS2*,
34
35 277 *qacA/qacC* and *ACME-arc* among colonising nasal staphylococci in individuals with no
36
37 278 previous healthcare exposure with greater prevalence among CoNS than *S. aureus*.
38
39 279 This pattern among pre-clinical medical students, supports CoNS as a reservoir with
40
41 280 potential to subsequently accelerate antimicrobial resistance and pathogenicity among
42
43 281 colonizing *S. aureus* in clinical environments under antibiotic selective pressure^{16 18 19}.

44
45 282 Despite considerable geographical distribution of the participants in this study, a
46
47 283 *S. aureus* nasal carriage rate in the community of 30.8% was recorded. In this study,
48
49 284 CC30, CC88 and CC8 were the most prevalent clones identified among nasal *S.*
50
51 285 *aureus*. CC30 is among the internationally disseminated clones in which *SCCmec* has
52
53 286 been acquired and is a successful colonising lineage, reported among HA and CA-
54
55 287 MRSA. Among medical students, these MSSA isolates may therefore represent a

1
2
3 288 significant pool for the uptake of *SCCmec* in a clinical setting. CC88 is frequently
4
5 289 isolated in Australia but in our study the geographical background of isolates was
6
7 290 mixed (including Middle East, Europe, South East Asia and Central America). CC8 is
8
9 291 associated with MRSA infection and is globally disseminated²⁰. Although CC30, CC88
10
11 292 and CC8 were prevalent among community MSSA isolates in this study, among the
12
13 293 relatively few MRSA recovered, none belonged to these CCs. Two CC/ST types
14
15 294 detected among MRSA recovered from healthy medical students in this study were
16
17 295 ST59-MRSA-IV and CC1-MRSA-V-*SCCfusC*. ST59 (Western Australian-MRSA-73) is
18
19 296 a sporadic Australian strain and apart from PVL-negativity, is indistinguishable from
20
21 297 USA1000²¹. In this study the geographical background of these isolates was wide
22
23 298 (Middle East, North America and South East Asia).

24
25 299 The identification of a significant reservoir of antibiotic resistance among
26
27 300 medical students prior to healthcare exposure in subsequent clinical years, highlights
28
29 301 the need for effective infection prevention and control policies in relation to hand
30
31 302 hygiene and surveillance. In the absence of antibiotic selective pressure, the colonising
32
33 303 MRSA rate appears relatively stable and in this study was 2 % (9/444), similar to rates
34
35 304 reported elsewhere²². However, a previous study among medical interns in China
36
37 305 reported a nasal MRSA rate of 9.4% likely reflecting exposure to the healthcare
38
39 306 environment²³. One study reported an increasing in carriage rates of MR-CoNS from
40
41 307 14% among medical student pre-internship, to 29.28% among interns²⁴. Prevalence
42
43 308 rates of MR-CoNS in recent community-based surveys are variable but rates of 16.5%
44
45 309¹⁹ and 17.2%²⁵ are reported in similar cohorts to this study where, of those colonised
46
47 310 with *S. aureus*, 13.1% carried MR-CoNS. *SCCmec* type IV, the smallest of the
48
49 311 *SCCmec* elements, was the most prevalent type among MRSA and MR-CoNS here
50
51 312 (66.6% and 50%). *SCCmec* IV has been detected in approximately 40% of methicillin-
52
53 313 resistant *S. epidermidis* identified in humans²⁶. However, in this study *SCCmec* type V
54
55 314 was also represented among MRSA and MR-CoNS. While only one individual was
56
57
58
59
60

1
2
3 315 colonised with MRSA and MR-CoNS in this study (both SCC*mec* type IV), the
4
5 316 preponderance of SCC*mec* IV element in nasal MRSA and MR-CoNS suggests the
6
7 317 potential for *mecA* gene transfer among these species even in the absence of selective
8
9 318 pressure. The small size of this element, which has a low fitness cost, may enhance its
10
11 319 dissemination potential.²⁷

12
13 320 Fusidic acid resistance among *S. aureus* from healthy carriers in nine European
14
15 321 countries in 2014 was reported to be <10 %²⁸. However, we found a prevalence of
16
17 322 22.6% of *fusC/fusB* genes among healthy carriers. Fusidic acid resistance appears to
18
19 323 correlate with increased use of this agent. For example, in New Zealand, where it is
20
21 324 used as a first-line empiric agent for topical treatment of impetigo, prevalence rates of
22
23 325 resistance in community *S. aureus* isolates increased from 17% in 1999 to 29% in 2013
24
25 326²⁹. In Europe, fusidic acid is combined with β -lactams for the treatment of
26
27 327 staphylococcal bacteraemia, endocarditis, and osteomyelitis³⁰ and is used widely in
28
29 328 the community for skin and soft tissue infections (SSTIs). A 2010 study of fusidic acid
30
31 329 resistance among *S. aureus* clinical isolates showed Greece and Ireland to have the
32
33 330 highest rates (52.5 and 19.9%)³¹. SCC*fus* has been identified in the CC1 background
34
35 331 and more recently, in other lineages such as ST239 and ST779³²⁻³⁴. As highlighted
36
37 332 here in the absence of significant antibiotic pressure in the community, it appears that
38
39 333 this element is associated with MRSA and MSSA in the CC1 background. This genetic
40
41 334 platform, particularly when associated with SCC*mec* in a composite element (SCC*mec*
42
43 335 V+SCC*fus*) may enable the transfer of multi-drug resistance in a single transfer event.
44
45 336 The use of fusidic acid is un-regulated in some countries and hence it may be used
46
47 337 inappropriately in a community setting (for example in short or discontinuous doses).
48
49 338 Inappropriate use of fusidic acid may therefore favour co-selection of methicillin-
50
51 339 resistance among *S. aureus*. In addition, in this study, 14/18 (77%) of MR-CoNS were
52
53 340 positive for *fusC* or *fusB*. This association of resistances among the resident flora may
54
55 341 provide further opportunity for dissemination of MRSA driven by fusidic acid selective

1
2
3 342 pressure. Interestingly a positive correlation between carriage of *fusC/fusB* and
4 343 phenotypic resistance was observed in only 71.9 % and 53.6 % of *S. aureus* and CoNS
5
6 344 respectively. However, induction of gene expression with fusidic acid pre-incubation
7
8 345 gave better correlation (82.2% and 76.3 % correlation).
9

10
11 346 There were limitations to this study, which included; a single centred, relatively
12
13 347 small study. Some nasally abundant CoNS species, for example *Staphylococcus*
14
15 348 *lugdunensis* and *Staphylococcus hominis*, were under-represented as the enrichment
16
17 349 method favoured pathogenic staphylococci such as *S. aureus* and *S. epidermidis*.
18
19 350 CoNS was investigated only in those co-colonised with *S. aureus* and therefore
20
21 351 prevalence rates for genes among CoNS do not reflect the entire cohort. CCs and STs
22
23 352 were determined only among *S. aureus* as the high rate of genetic recombination
24
25 353 among CoNS makes strain typing unreliable. Although the microarray system used is
26
27 354 reported effective for staphylococcal species other than *S. aureus*³⁵, some gene
28
29 355 targets may be heterologous among staphylococci leading to false negatives. The
30
31 356 study design did not facilitate follow-up of this cohort during clinical training which may
32
33 357 have revealed further changes in gene carriage among colonising staphylococci.
34
35 358 However, the multi-national origin of the student body in our institution facilitated
36
37 359 analysis of a relatively broad geographic cohort in a single study and emphasises the
38
39 360 role that importation plays in *S. aureus* epidemiology. Unlike other studies of
40
41 361 staphylococci in the healthy human nares, pairs of staphylococcal species originating
42
43 362 from the same individual were investigated here for their resistance and virulence traits.
44
45 363 These data support a low rate of transfer of antibiotic resistance between colonising
46
47 364 staphylococcal species in the absence of healthcare contact. However, it is concerning
48
49 365 that similar *SCCmec* and *SCCfusC* types, in addition to *ileS2*, *qacA/qacB* and ACME
50
51 366 are carried among CoNS and *S. aureus* in healthy individuals who will have
52
53 367 subsequent roles in healthcare provision. Given the increasing emergence of HA-
54
55 368 MRSA with features of community strains, further mobilisation of these elements under
56
57
58
59
60

1
2
3 369 selective antibiotic pressure may enhance the transmission and success of *S. aureus*
4
5 370 in the healthcare environment.

6
7 371 **Funding**

8
9
10 372 PEB received funding for the study from Coordenação de Aperfeiçoamento de Pessoal
11
12 373 de Nível Superior (CAPES), Brazil under the Science without Borders Program. Grant
13
14 374 number 9172-13-0.

15
16 375 **Competing interests**

17
18
19 376 HH has received funding from Pfizer and Astellas outside the relevance of the
20
21 377 submitted work, all other authors report no competing interests.

22
23 378 **Contributor Statement**

24
25
26 379 PEB and DFH recruited students to the study, PEB conducted the laboratory work and
27
28 380 drafted the manuscript. DFH and HH conceived of the study and contributed to study
29
30 381 design. AS, PK and DC provided critical data interpretation and revised the drafted
31
32 382 work. All authors contributed to the final approved draft.

33
34 383 **Data sharing statement**

35
36
37 384 All data for these analyses are included in the manuscript. No additional data are
38
39 385 available.

40
41 386 **Acknowledgements**

42
43
44 387 We acknowledge the support of the Mercer's medical centre, RCSI and Ms Helen
45
46 388 Barry, Chief Medical Scientist, St. James' Hospital Dublin.

47
48
49 389

50
51 390 **Figure Legends**

52
53
54 391 **Figure 1. Geographical origin of medical students recruited.** The geographical
55
56 392 areas of origin of 444 medical students recruited to the study are shown (dark grey

1
2
3 393 bars). Of those recruited, 137 were confirmed nasal *S. aureus* and CoNS positive. The
4
5 394 proportion of recruited students from each geographical origin with nasal *S. aureus*
6
7 395 carriage are also shown (light grey bars).
8
9
10 396

11
12 397 **Figure 2.** Genotypic diversity of 137 *S. aureus* nasal isolates using DNA microarray
13
14 398 analysis, including 128 MSSA (dark grey bars) and 9 MRSA (light grey bars). Letter (a)
15
16 399 indicates internationally disseminated clones into which SCCmec can integrate.
17
18 400 CC=clonal complex.
19
20
21 401
22
23 402
24
25 403
26
27 404
28
29 405
30
31 406
32
33 407
34
35 408
36
37 409
38
39 410
40
41 411
42
43 412
44
45 413
46
47 414
48
49
50
51
52
53
54
55
56
57
58
59
60

415

416

417

418

419

420

421

422

423

424

425

426

427

428

429

430

431

432

433

Table

1.

Staphy

lococc

us

specie

s

recove

red

from

444

nasal

swabs.

Staphylococcus species recovered

Total
n, (%)Methicillin-
resistant
phenotype
n, (%)

n=444

n=137^a*S. aureus*

137, (30.8)

MRSA

9, (6.6)

S. epidermidis

364, (81.9)

MRSE

17, (12.4)

S. haemolyticus

14, (3.1)

MRSH

0

S. saprophyticus

8, (1.8)

MRSS

1, (0.72)

Co-carriage species*S. aureus* + *S. epidermidis*

137, (30.8)

MRSA + MR-CoNS

1, (0.72)

fusC positive *S. aureus* + CoNS

1, (0.72)

^a CoNS were investigated only in those positive for nasal *S. aureus* in the student cohort and not in all those recruited. MRSA= methicillin resistant *S. aureus*, MRSE = methicillin resistant *S. epidermidis*, MRSH = methicillin resistant *S. haemolyticus*, MRSS = methicillin resistant *S. saprophyticus*

434 Table 2. Resistance/virulence genes detected among 137 co-located nasal *S. aureus*/CoNS pairs.

Detected Gene(s)	Phenotypic resistance/trait	No. isolates positive		<i>P</i> value	No. <i>S. aureus</i> /CoNS pairs positive (n)
		n (%)			
		<i>S. aureus</i> n=137	CoNS n=137		
<i>Antibiotic resistance gene</i>					
<i>blaZ</i>	β-lactam	101 (73.7)	92 (67.1)	0.289	74
<i>fusB</i>	Fusidic acid	2 (1.5)	27 (19.7)	0.0002*	0
<i>fusC^a</i>	Fusidic acid	31 (22.6)	25 (18.2)	0.159	1
<i>mecA</i>	Methicillin	9 (6.5)	18 (13.1)	0.103	1
<i>ileS2</i>	Mupirocin	1 (0.7)	11 (8.0)	0.005*	1
<i>qacA</i> and <i>qacC</i>	Quaternary ammonium salts	3 (2.2)	29 (21.2)	<0.0001*	0
<i>tet(K)</i> and <i>tet(M)</i>	Tetracycline	13 (9.5)	6 (4.4)	0.152	0
<i>erm(C)</i>	Macrolide/lincosamide	6 (4.3)	5 (3.6)	1.000	0
<i>msr(A)</i>	Macrolide	2 (1.45)	15 (10.9)	0.002*	1
<i>mph(C)</i>	Macrolide	0	15 (10.9)	<0.0001*	0
<i>dfrS1</i>	Trimethoprim	0	19 (13.8)	<0.0001*	0

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

<i>vga</i>	Streptogramin A	1 (0.7)	6 (4.3)	0.120	0
<i>Virulence</i>					
ACME- <i>arc</i>	pH tolerance	1 (0.7)	44 (32.1)	<0.0001*	1
<i>tst1^b</i>	Toxic shock toxin	33 (24.1)	2 (1.5)	<0.0001*	0

435 ^aassociated with SCC element, (*ccrA-1* and *ccrB-1*) in 13/137 *S. aureus*. ^b*tst1* confirmed by PCR. ACME = Arginine Catabolite Mobile Element. * indicates a
 436 statistically significant result by Fisher's exact test.

437

For peer review only

1
2
3
4
5
6 438
7
8
9
10
11
12
13
14
15
16
17
18
19 439
20
21 440
22
23 441
24
25 442
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

Table 3. Fusidic acid MICs for *S. aureus* and CoNS

	MIC ≤ 1 µg/ml	MIC ≥ 1 µg/ml	MIC ≥ 32 µg/ml
MIC Interpretation ^a	S	R	HR
	n (%)	n(%)	n (%)
<i>S. aureus</i> (n = 32)	9 (28.1)	20 (62.5)	3 (9.3)
CoNS (n = 38)	18 (47.4)	15 (39.5)	5 (13.2)

^aInterpretation based on The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017. <http://www.eucast.org>, S = Susceptible, R = Resistant, HR = high level resistant

443 **References**

444

- 445 1. Fluit AC, Carpaij N, Majoor EA, et al. Shared reservoir of *ccrB* gene sequences
446 between coagulase-negative staphylococci and methicillin-resistant
447 *Staphylococcus aureus*. *The Journal of antimicrobial chemotherapy*
448 2013;**68**(8):1707-13. doi: 10.1093/jac/dkt121 [published Online First:
449 2013/04/20]
- 450 2. Shore AC, Coleman DC. Staphylococcal cassette chromosome mec: recent
451 advances and new insights. *International journal of medical microbiology : IJMM*
452 2013;**303**(6-7):350-9. doi: 10.1016/j.ijmm.2013.02.002 [published Online First:
453 2013/03/19]
- 454 3. Hung WC, Chen HJ, Lin YT, et al. Skin Commensal Staphylococci May Act as
455 Reservoir for Fusidic Acid Resistance Genes. *PLoS One*
456 2015;10(11):e0143106. doi: 10.1371/journal.pone.0143106 [published Online
457 First: 2015/11/19]
- 458 4. Ellington MJ, Reuter S, Harris SR, et al. Emergent and evolving antimicrobial
459 resistance cassettes in community-associated fusidic acid and methicillin-
460 resistant *Staphylococcus aureus*. *International journal of antimicrobial agents*
461 2015;**45**(5):477-84. doi: 10.1016/j.ijantimicag.2015.01.009 [published Online
462 First: 2015/03/15]
- 463 5. Baines SL, Howden BP, Heffernan H, et al. Rapid Emergence and Evolution of
464 *Staphylococcus aureus* Clones Harboring *fusC*-Containing Staphylococcal
465 Cassette Chromosome Elements. *J Antimicrob Chemother* 2016;**60**(4):2359-65.
466 doi: 10.1128/aac.03020-15
- 467 6. Miragaia M, de Lencastre H, Perdreau-Remington F, et al. Genetic diversity of
468 arginine catabolic mobile element in *Staphylococcus epidermidis*. *PLoS One*

- 1
2
3 469 2009;**4**(11):e7722. doi: 10.1371/journal.pone.0007722 [published Online First:
4 470 2009/11/07]
5
6
7 471 7. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an
8 472 epidemic clone of community-acquired methicillin-resistant *Staphylococcus*
9 473 *aureus*. *Lancet* 2006;**367**(9512):731-9. doi: S0140-6736(06)68231-7 [pii]
10
11
12
13 474 10.1016/S0140-6736(06)68231-7 [doi] [published Online First: 2006/03/07]
14
15 475 8. Gonzalez-Dominguez M, Seral C, Potel C, et al. Genotypic and phenotypic
16 476 characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) clones
17 477 with high-level mupirocin resistance. *Diagn Microbiol Infect Dis* 2016;**85**(2):213-
18
19 478 7. doi: 10.1016/j.diagmicrobio.2016.02.021 [published Online First: 2016/05/03]
20
21
22
23 479 9. Bathoorn E, Hetem DJ, Alphenaar J, et al. Emergence of high-level mupirocin
24 480 resistance in coagulase-negative staphylococci associated with increased
25 481 short-term mupirocin use. *J Clin Microbiol* 2012;**50**(9):2947-50. doi:
26
27 482 10.1128/jcm.00302-12 [published Online First: 2012/07/05]
28
29
30
31 483 10. Earls MR, Kinnevey PM, Brennan GI, et al. The recent emergence in hospitals of
32 484 multidrug-resistant community-associated sequence type 1 and spa type t127
33 485 methicillin-resistant *Staphylococcus aureus* investigated by whole-genome
34
35 486 sequencing: Implications for screening. *PloS one* 2017;**12**(4):e0175542. doi:
36
37 487 10.1371/journal.pone.0175542
38
39
40
41 488 11. Huber H, Giezendanner N, Stephan R, et al. Genotypes, antibiotic resistance
42 489 profiles and microarray-based characterization of methicillin-resistant
43 490 *Staphylococcus aureus* strains isolated from livestock and veterinarians in
44
45 491 Switzerland. *Zoonoses and public health* 2011;**58**(5):343-9. doi: 10.1111/j.1863-
46
47 492 2378.2010.01353.x [published Online First: 2010/09/21]
48
49
50
51 493 12. Monecke S, Jatzwauk L, Weber S, et al. DNA microarray-based genotyping of
52 494 methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. *Clin*
53
54 495 *Microbiol Infect* 2008;**14**(6):534-45. doi: CLM1986 [pii]
55
56
57
58
59
60

- 1
2
3 496 10.1111/j.1469-0691.2008.01986.x [doi] [published Online First: 2008/04/01]
4
5 497 13. Monecke S, Slickers P, Ehricht R. Assignment of *Staphylococcus aureus* isolates to
6
7 498 clonal complexes based on microarray analysis and pattern recognition. *FEMS*
8
9 499 *Immunol Med Microbiol* 2008;**53**(2):237-51. doi: FIM426 [pii]
10
11 500 10.1111/j.1574-695X.2008.00426.x [doi] [published Online First: 2008/05/30]
12
13 501 14. O'Neill AJ, Larsen AR, Henriksen AS, et al. A fusidic acid-resistant epidemic strain
14
15 502 of *Staphylococcus aureus* carries the *fusB* determinant, whereas *fusA*
16
17 503 mutations are prevalent in other resistant isolates. *Antimicrobial agents and*
18
19 504 *chemotherapy* 2004;**48**(9):3594-7. doi: 10.1128/aac.48.9.3594-3597.2004
20
21 505 [published Online First: 2004/08/26]
22
23 506 15. Chen HJ, Hung WC, Tseng SP, et al. Fusidic acid resistance determinants in
24
25 507 *Staphylococcus aureus* clinical isolates. *Antimicrobial agents and*
26
27 508 *chemotherapy* 2010;**54**(12):4985-91. doi: 10.1128/aac.00523-10 [published
28
29 509 Online First: 2010/09/22]
30
31 510 16. Iravani Mohammad Abadi M, Moniri R, Khorshidi A, et al. Molecular Characteristics
32
33 511 of Nasal Carriage Methicillin-Resistant Coagulase Negative Staphylococci in
34
35 512 School Students. *Jundishapur journal of microbiology* 2015;**8**(6):e18591. doi:
36
37 513 10.5812/jjm.18591v2 [published Online First: 2015/08/25]
38
39 514 17. Faria NA, Conceicao T, Miragaia M, et al. Nasal carriage of methicillin resistant
40
41 515 staphylococci. *Microb Drug Resist* 2014;**20**(2):108-17. doi:
42
43 516 10.1089/mdr.2013.0197 [published Online First: 2014/02/26]
44
45 517 18. Jamaluddin TZ, Kuwahara-Arai K, Hisata K, et al. Extreme genetic diversity of
46
47 518 methicillin-resistant *Staphylococcus epidermidis* strains disseminated among
48
49 519 healthy Japanese children. *Journal of clinical microbiology* 2008;**46**(11):3778-
50
51 520 83. doi: 10.1128/jcm.02262-07 [published Online First: 2008/10/04]
52
53 521 19. Barbier F, Ruppe E, Hernandez D, et al. Methicillin-resistant coagulase-negative
54
55 522 staphylococci in the community: high homology of SCCmec IVa between
56
57
58
59
60

- 1
2
3 523 *Staphylococcus epidermidis* and major clones of methicillin-resistant
4 524 *Staphylococcus aureus*. *J Infect Dis* 2010;**202**(2):270-81. doi: 10.1086/653483
5
6 525 [doi] [published Online First: 2010/06/17]
7
8 526 20. Jimenez JN, Ocampo AM, Vanegas JM, et al. CC8 MRSA strains harboring
9 527 SCCmec type IVc are predominant in Colombian hospitals. *PloS one*
10 528 2012;**7**(6):e38576. doi: 10.1371/journal.pone.0038576 [published Online First:
11 529 2012/06/30]
12
13 530 21. Monecke S, Coombs G, Shore AC, et al. A field guide to pandemic, epidemic and
14 531 sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PloS one*
15 532 2011;**6**(4):e17936. doi: 10.1371/journal.pone.0017936 [published Online First:
16 533 2011/04/16]
17
18 534 22. Abroo S, Hosseini Jazani N, Sharifi Y. Methicillin-resistant *Staphylococcus aureus*
19 535 nasal carriage between healthy students of medical and nonmedical
20 536 universities. *American journal of infection control* 2017;**45**(7):709-12. doi:
21 537 10.1016/j.ajic.2017.02.034 [published Online First: 2017/04/01]
22
23 538 23. Ma XX, Sun DD, Wang S, et al. Nasal carriage of methicillin-resistant
24 539 *Staphylococcus aureus* among preclinical medical students: epidemiologic and
25 540 molecular characteristics of methicillin-resistant *S. aureus* clones. *Diagnostic*
26 541 *microbiology and infectious disease* 2011;**70**(1):22-30. doi:
27 542 10.1016/j.diagmicrobio.2010.12.004 [published Online First: 2011/04/26]
28
29 543 24. Baragundi MC, Solabannavar SS, Gokale SK, et al. Methicillin and multidrug
30 544 resistant coagulase negative staphylococcal nasal carriage in medical students.
31 545 *The Journal of communicable diseases* 2012;**44**(4):231-7. [published Online
32 546 First: 2012/12/01]
33
34 547 25. Du X, Zhu Y, Song Y, et al. Molecular analysis of *Staphylococcus epidermidis*
35 548 strains isolated from community and hospital environments in China. *PloS one*
36 549 2013;**8**(5):e62742. doi: 10.1371/journal.pone.0062742 [published Online First:
37 550 2013/05/16]
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 551 26. Miragaia M, Thomas JC, Couto I, et al. Inferring a population structure for
4
5 552 *Staphylococcus epidermidis* from multilocus sequence typing data. *Journal of*
6
7 553 *bacteriology* 2007;**189**(6):2540-52. doi: 10.1128/jb.01484-06 [published Online
8
9 554 First: 2007/01/16]
- 10
11 555 27. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant
12
13 556 *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA
14
15 557 strains? *Clinical infectious diseases: an official publication of the Infectious*
16
17 558 *Diseases Society of America* 2008;**46**(6):787-94. doi: 10.1086/528716
18
19 559 [published Online First: 2008/02/13]
- 20
21 560 28. den Heijer CD, van Bijnen EM, Paget WJ, et al. Fusidic acid resistance in
22
23 561 *Staphylococcus aureus* nasal carriage strains in nine European countries.
24
25 562 *Future Microbiol* 2014;**9**(6):737-45. doi: 10.2217/fmb.14.36 [published Online
26
27 563 First: 2014/07/22]
- 28
29 564 29. Williamson DA, Monecke S, Heffernan H, et al. High usage of topical fusidic acid
30
31 565 and rapid clonal expansion of fusidic acid-resistant *Staphylococcus aureus*: a
32
33 566 cautionary tale. *Clinical infectious diseases: an official publication of the*
34
35 567 *Infectious Diseases Society of America* 2014;**59**(10):1451-4. doi:
36
37 568 10.1093/cid/ciu658 [published Online First: 2014/08/21]
- 38
39 569 30. Whitby M. Fusidic acid in septicaemia and endocarditis. *International journal of*
40
41 570 *antimicrobial agents* 1999;**12**:S17-S22. doi: [http://dx.doi.org/10.1016/S0924-](http://dx.doi.org/10.1016/S0924-8579(98)00070-3)
42
43 571 [8579\(98\)00070-3](http://dx.doi.org/10.1016/S0924-8579(98)00070-3)
- 44
45 572 31. Castanheira M, Watters AA, Mendes RE, et al. Occurrence and molecular
46
47 573 characterization of fusidic acid resistance mechanisms among *Staphylococcus*
48
49 574 spp. from European countries (2008). *J Antimicrob Chemother*
50
51 575 2010;**65**(7):1353-8. doi: 10.1093/jac/dkq094 [published Online First:
52
53 576 2010/05/01]
- 54
55 577 32. Holden MT, Feil EJ, Lindsay JA, et al. Complete genomes of two clinical
56
57 578 *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and

- 1
2
3 579 drug resistance. *Proc Natl Acad Sci U S A* 2004;**101**(26):9786-91. doi:
4 580 10.1073/pnas.0402521101 [published Online First: 2004/06/24]
5
6 581 33. Kinnevey PM, Shore AC, Brennan GI, et al. Emergence of sequence type 779
7
8 582 methicillin-resistant *Staphylococcus aureus* harboring a novel pseudo
9
10 583 staphylococcal cassette chromosome mec (SCCmec)-SCC-SCCCRISPR
11
12 584 composite element in Irish hospitals. *Antimicrob Agents Chemother*
13
14 585 2013;**57**(1):524-31. doi: 10.1128/aac.01689-12 [published Online First:
15
16 586 2012/11/14]
17
18 587 34. Lin YT, Tsai JC, Chen HJ, et al. A novel staphylococcal cassette chromosomal
19
20 588 element, SCCfusC, carrying *fusC* and *speG* in fusidic acid-resistant methicillin-
21
22 589 resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*
23
24 590 2014;**58**(2):1224-7. doi: 10.1128/aac.01772-13 [published Online First:
25
26 591 2013/11/28]
27
28 592 35. Argudin MA, Vanderhaeghen W, Butaye P. Diversity of antimicrobial resistance and
29
30 593 virulence genes in methicillin-resistant non-*Staphylococcus aureus*
31
32 594 staphylococci from veal calves. *Research in veterinary science* 2015;**99**:10-6.
33
34 595 doi: 10.1016/j.rvsc.2015.01.004 [published Online First: 2015/02/01]
35
36
37 596
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

597

598

For peer review only

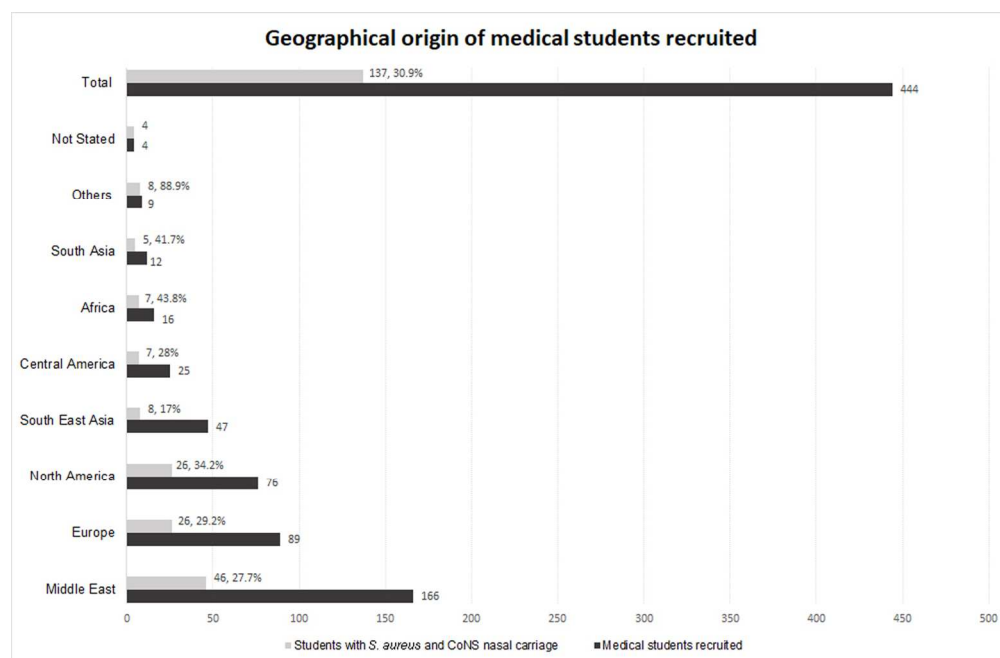


Figure 1. Geographical origin of medical students recruited. The geographical areas of origin of 444 medical students recruited to the study are shown (dark grey bars). Of those recruited, 137 were confirmed nasal *S. aureus* and CoNS positive. The proportion of recruited students from each geographical origin with nasal *S. aureus* carriage are also shown (light grey bars).

147x95mm (300 x 300 DPI)

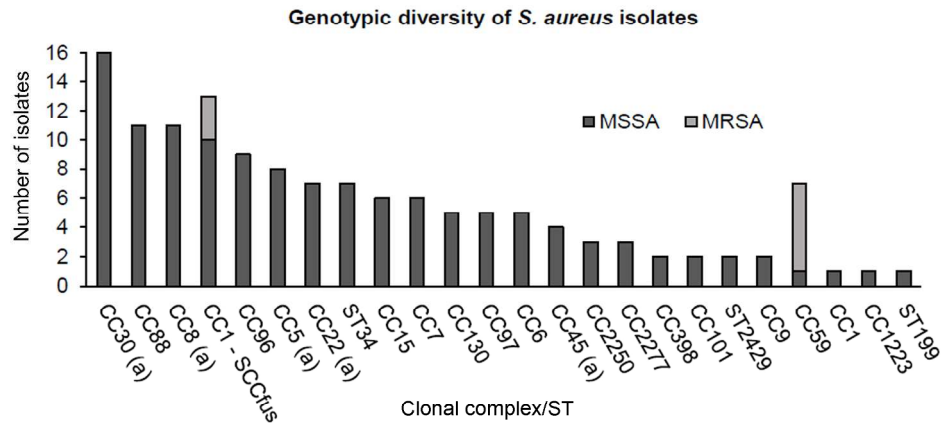


Figure 2. Genotypic diversity of 137 *S. aureus* nasal isolates using DNA microarray analysis, including 128 MSSA (dark grey bars) and 9 MRSA (light grey bars). Letter (a) indicates internationally disseminated clones into which SCC_{mec} can integrate. CC=clonal complex.

222x127mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Relevant lines in manuscript and confirmation of items
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1- Title 1-Abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Yes
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Yes
Objectives	3	State specific objectives, including any prespecified hypotheses	25-29 (Abstract) 93-97 (Introduction)
Methods			
Study design	4	Present key elements of study design early in the paper	100
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	99-113
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	103-106
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	n/a n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	n/a
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	134-138 (Identification of genes) 146-149 (fusidic acid susceptibility)

			110-111 (participant details, age range, region of origin, healthcare exposure)
Bias	9	Describe any efforts to address potential sources of bias	102-106 (anonymous data collection, high participation rate no healthcare contact reported etc)
Study size	10	Explain how the study size was arrived at	105
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	152-154 (statistical analyses of variables)
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	152-154
		(b) Describe any methods used to examine subgroups and interactions	n/a
		(c) Explain how missing data were addressed	n/a
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	n/a
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	n/a
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	n/a
		(e) Describe any sensitivity analyses	

Continued on next page

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	105, 158
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	164-165 (relevant demographic region of origin)
		(b) Indicate number of participants with missing data for each variable of interest	n/a
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	n/a
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	n/a
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	n/a
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	158, 159 <i>S. aureus</i> and MRSA colonisation 162-163 MRSE among <i>S. aureus</i> colonised 163-163 Co-carriage of MRSA MRSE
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	179-183 SCCmec types among MRSA 183-186 SCCmec types among CoNS 189-194 fusC carriage among <i>S. aureus</i> and CoNS 200-201 Co-carriage of fus C 205-209 fusidic acid resistance among <i>S. aureus</i> /CoNS Confounders – n/a
		(b) Report category boundaries when continuous variables were categorized	n/a
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	249-254 257-260 285-288 300-303
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both	330-341

		direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	YES
Generalisability	21	Discuss the generalisability (external validity) of the study results	285-290 – comparison to other studies, stability of findings in relations to carriage rates
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	356-358

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.