European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:927–936
https://doi.org/10.1007/s10096-019-03523-0

ORIGINAL ARTICLE

A molecular epidemiological investigation of methicillin-susceptible *Staphylococcus aureus* causing bloodstream infections in Ireland, 2006–2017

Emily C. Deasy[1] · **Gráinne I. Brennan**[2] · **Sarah C. Tecklenborg**[1] · **Chioma Umeh**[1] · **David C. Coleman**[1] · **Anna C. Shore**[1]

Received: 5 December 2018 / Accepted: 27 February 2019 / Published online: 23 March 2019

Abstract

The prevalence of methicillin-susceptible *Staphylococcus aureus* (MSSA) bloodstream infections (BSIs) has increased in many countries, including Ireland. This study aimed to investigate the molecular epidemiology of MSSA causing BSIs in Irish hospitals between 2006 and 2017, when MSSA BSIs increased, to identify any potential patient or pathogen contributing factors. A total of 252 MSSA isolates from patients in Irish hospitals in 2006/2007, 2011 and 2017 underwent *spa* typing and DNA microarray profiling. Each patient's gender, age, 14-day mortality and epidemiological context of infection were recorded. Significant increases in community-onset (CO) MSSA BSIs and the average patient's age and decreases in hospital-onset (HO) MSSA were identified. Although, extensive genetic diversity was detected amongst the isolates, i.e. 24 multilocus sequence type clonal complexes (CCs)/sequence types and 124 *spa* types, three CCs (CC30, CC45, CC5) dominated, albeit in different proportions, during the study periods. CC30 declined significantly, in particular *spa* type t021, and was more common amongst HO-MSSA and CC45 and CC8 increased, particularly *spa* types t015 and t008, respectively, and were more common amongst CO-MSSA. Five of the seven most frequent *spa* types were more common amongst CO-MSSA. Although overall multidrug resistance decreased, the prevalence of *erm*(C) increased significantly and virulence genes decreased, mostly notably *egc*, *tst*, *scn*, *sep* and *fnbB*. This study highlights the threat posed by the increasing prevalence of CO-MSSA BSIs and suggests an association with the increasing prevalence of CC45 CO-MSSA, decreasing prevalence of CC30 HO-MSSA, an ageing population and an overall decrease in virulence and resistance genes.

Keywords MSSA · Bloodstream infections · Molecular epidemiology · Virulence genes · Resistance genes

Introduction

Bloodstream infections (BSIs) are one of the most serious forms of healthcare-associated infections (HCAIs) and are associated with high morbidity and mortality, imposing a substantial burden on patients and healthcare systems [1]. Globally, *Staphylococcus aureus* is one of the most frequently isolated pathogens from BSIs [2–4]. Although, methicillin-resistant *S. aureus* (MRSA) have been linked to more persistent BSIs than methicillin-susceptible *S. aureus* (MSSA) and with a higher mortality rate [5, 6], some studies have reported comparable, and in some instances increasing, mortality rates for MSSA BSIs [7–9].

Annually in Ireland, *S. aureus* accounted for approximately 19–32% of BSIs reported from Irish hospitals between 2006 and 2016 and was consistently the second most common cause of BSIs, second only to *Escherichia coli* [2]. The proportion of MRSA amongst *S. aureus* BSIs in Ireland decreased significantly from 41.9% in 2006 to 14.7% in 2016, and this was also reflected in a decrease in the MRSA BSI rate for acute hospitals from 0.147 to 0.043 cases per 1000 bed days used (BDU). Conversely, between 2006 and 2016 the proportion of MSSA increased from 58.1 to 85.3% of

---

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10096-019-03523-0) contains supplementary material, which is available to authorized users.

✉ Anna C. Shore
anna.shore@dental.tcd.ie

[1] Microbiology Research Unit, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Dublin, Ireland

[2] National MRSA Reference Laboratory, St. James's Hospital, Dublin, Ireland

S. aureus BSIs and from 0.206 to 0.245 cases per 1000 BDU [10]. Similar trends have been observed in hospitals across Europe with increasing rates of *S. aureus* BSIs associated with declining rates of MRSA BSIs but a steady incline amongst rates of MSSA BSIs [4, 11, 12].

Two European-wide studies were carried out in 2006/2007 and 2011 and investigated the *spa* types of MRSA and MSSA BSI isolates in Europe [12, 13]. These studies revealed that MSSA BSI isolates are more genetically diverse without any dominant geographical pattern in comparison to MRSA, the latter of which occur in distinct geographical clusters with significantly less diversity. There was little difference, apart from rank order, in the top *spa* types amongst MRSA and MSSA across the entire region during both study periods. Amongst the MRSA isolates, those exhibiting the ST22-MRSA-IV *spa* type t032 (UK EMRSA-15), predominated, particularly in Ireland and the UK. Several studies have demonstrated the predominance of ST22-MRSA-IV for the last two decades in both of these countries, despite declining MRSA rates [14–16]. Amongst the MSSA isolates in the European *spa* typing studies, the rank order of the most common *spa* types changed but little difference was seen in the predominant *spa* types in 2006 and 2011 [13]. These studies also revealed that MSSA was more common in younger patients, had a lower mortality rate and was less likely to be HCA than MRSA, with an increase in the age distribution, 14-day mortality rates and community-onset (CO) infections in 2011 compared to 2006 for both MRSA and MSSA [12, 13]. Another study indicated that MSSA results in more serious infections than MRSA, and speculated that this may be due to a fitness cost imposed by SCC*mec* in MRSA or the higher prevalence of virulence genes amongst MSSA [17].

With the increasing prevalence of MSSA infections, in particular serious and life-threatening BSIs, detailed molecular epidemiological studies are required. Although the Grundmann et al. studies [12, 13] investigated the *spa* types and patient data of MSSA from BSIs at a European-wide level in 2006/2007 and 2011, very few studies have investigated this at a national level. Studies that focussed solely on MSSA and on BSIs and analysed isolates recovered over long periods of time to identify temporal changes or that analysed virulence and resistance genes in addition to genotypes are scarce [18–24]. Recent data on the molecular characteristics of MSSA from BSIs in individual European countries is particularly meagre [20]. Therefore, this study aimed to describe the molecular epidemiology of MSSA causing BSIs amongst patients in Irish hospitals over the 11-year period from 2006 to 2017 when MSSA BSIs increased, and to determine if any change in MSSA virulence factor(s), antimicrobial resistance gene(s), clonal type, or patient demographic(s) could be attributed to the observed increase in MSSA BSIs.

Material and methods

Isolates investigated

A total of 252 MSSA isolates recovered from patients with BSIs in Irish hospitals over 6-month periods in 2006/2007, 2011 and 2017 were investigated. This included 84 MSSA isolates recovered between September 2006 and February 2007, 98 recovered between January and June 2011 and 70 recovered between January and June 2017. The isolates were recovered from patients in 21, 23 and 20 hospitals across Ireland that participated in EARS-Net in 2006/2007, 2011 and 2017, respectively. Only one isolate per patient was investigated and up to a maximum of five isolates from each hospital, these being the first five consecutive MSSA isolates recovered from each hospital during the study period. The 2006 and 2011 isolates were collected as part of two European *spa* typing studies [12, 13]. The collection of all isolates was coordinated via the Irish National MRSA Reference Laboratory.

Patient data was collected for each isolate and included gender, age, the all-cause mortality 14 days after the first isolation of MSSA and the epidemiological context of the infection, i.e. community-onset (CO) infection (symptoms present upon or within 48 h of admission) or hospital-onset (HO) infection (symptoms developed more than 48 h following admission). Isolates were confirmed as *S. aureus* using the tube coagulase test and by DNA microarray profiling (see below). Isolates were stored at − 80 °C on cryoprotective beads (Technical Service Consultants Ltd., UK). Susceptibility to methicillin was confirmed using cefoxitin disks (30 μg) (Oxoid Ltd., Basingstoke, UK) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology and interpretive criteria [25].

Molecular characterisation of isolates

Total genomic DNA was extracted from all isolates by enzymatic lysis using the buffers and solutions provided with the *S. aureus* Genotyping Kit 2.0 ([Abbott] Alere Technologies GmbH, Jena, Germany) and the DNeasy Blood and Tissue Kit (Qiagen, Crawley, West Sussex, UK) and served as a template for *spa* typing and DNA microarray profiling.

spa typing was performed using the primers and thermal cycling conditions described by the European Network of Laboratories for Sequence Based Typing of Microbial Pathogens (SeqNet; www.seqnet.org.). *spa* typing PCR products were purified with the GenElute PCR Clean-Up Kit ([Merck] Sigma-Aldrich Ireland Ltd., Arklow, County Wicklow, Ireland) and sequencing was performed commercially by Source Bioscience (Tramore, Waterford, Ireland). The Ridom StaphType software versions 1.3 and 1.5 (Ridom

GmbH, Wurzburg, Germany) were used for *spa* sequence analysis and assignment of *spa* types.

The *S. aureus* Genotyping Kit 2.0 ([Abbott]Alere Technologies, GmbH) was used for DNA microarray profiling to assign *S. aureus* isolates to MLST clonal complexes (CCs) and sequence types (STs) and to detect a range of species-specific and typing markers and virulence-associated and antimicrobial resistance genes. The DNA microarray primers, probes and protocols have been described previously in detail [26].

Comparison of patient and isolate data

The comparative analysis of all data was performed using the Microsoft Excel software package (version 14.0). Patient data, *spa* types, CCs/STs and virulence and antimicrobial resistance gene carriage were compared for all MSSA isolates across the three study periods in pairwise combinations, i.e. 2006/2007 versus 2011, 2006/2007 versus 2017 and 2011 versus 2017. Statistical analysis was performed using GraphPad QuickCalcs (https://www.graphpad.com/quickcalcs/). A two-tailed Fisher's exact test was used to determine if differences were significant. For numerical data, i.e. age of patients during the different study periods, statistical analysis was performed using a Mann-Whitney *U* test (data was not parametric) in GraphPad Prism (version 7). For all statistical analyses, results were considered significant if the tests resulted in a p value < 0.05, at a 95% confidence level.

Results

Patient demographics

The incidence of CO-MSSA BSIs increased and HO-MSSA decreased across the three study periods with the increase in CO-MSSA from 42% in 2006/2007 to 70% in 2017 ($p = 0.0016$) and the decreases in HO-MSSA from 58% in 2006/2007 to 30% in 2017 ($p = 0.0016$) and from 47% in 2011 to 30% in 2017 ($p = 0.0473$) being significant (Table 1). The proportion of males with MSSA (63–64%) and the all-cause mortality 14 days after the first isolation of MSSA (7–10%) were similar across the three studies periods (Table 1) as was the 14-day mortality rate for HO- (9.9%, 10/101) and CO-MSSA (9.0%, 11/122). The average age of the patients with MSSA BSIs increased across the three study periods from 51.7 years (range 3 days to 89 years) in 2006/2007 to 54.9 years (range 2 months to 91 years) in 2011 and 61.2 years (age range 3 to 90 years) in 2017 (Table 1), with the increase between 2006/2007 and 2017 being significant ($p = 0.0357$).

Genotyping

The 252 MSSA isolates were assigned to 24 MLST CCs/STs and 124 *spa* types. There was a slight but non-significant increase in the CC/ST per isolate ratio across the three study periods, i.e. 0.19 in 2006/2007 (16 CCs/STs amongst 84 isolates), 0.20 in 2011 (20 CCs/STs amongst 98 isolates) and 0.23 in 2017 (16 CCs/STs amongst 70 isolates), although the *spa* type per isolate ratios were similar (0.64, 54/84 in 2006/2007; 0.65, 64/98 in 2011; 0.61, 43/70 in 2017). The MLST CCs 30 (15.5%, 39/252), 45 (18.7%, 47/252) and 5 (11.9%, 30/252) predominated and were the dominant clones during all three study periods. The most common *spa* types were t002 (4.8%, 12/252), t008 (4.8%, 12/252), t012 (4.4%, 11/252), t091 (4.4%, 11/252), t127 (4.4%, 11/252), t021 (4%, 10/252) and t230 (4%, 10/252). Worth noting was the significant decrease ($p = 0.0164$) in the prevalence of CC30 between 2006 (23.8%, 20/84) and 2011 (10.2%, 10/98) (Fig. 1a), in particular those exhibiting *spa* type t021 and the increase in the prevalence of CC45 and CC8, particularly t015 and t008, respectively, across the study periods. Whilst there were no significant associations between the epidemiological context of the infection, i.e. CO-/HO-MSSA and MLST-CC/ST, 63.8% (30/47) of CC45 isolates were CO-MSSA, accounting for 40% (6/15) of CC45 isolates in 2006/2007 and increasing to 73.3% (11/15) and 76.5% (13/17), in 2011 and 2017, respectively. Conversely, 58.8% (20/34) of CC30 isolates were HO-MSSA, accounting for 29.4% (10/34) of CC30 isolates in 2006/2007 but decreasing to 17.7% (6/34) and 11.8% (4/34) in 2011 and 2017, respectively. Interestingly, 5/7 top *spa* types were more common amongst CO-MSSA including t002 (8/12), t008 (7/12), t091 (7/11), t127 (5/7) and t230 (6/9).

Antimicrobial resistance and virulence genes

Antimicrobial resistance genes The most common antimicrobial resistance gene was *blaZ* (Fig. 2a). The antimicrobial resistance genes *erm*(A), *erm*(C), *aacA-aphD*, *fusC* and *tet*(K) were also detected in small numbers of isolates, in all three study periods (Fig. 2a). The increase in *erm*(C) from 2.0% (2/98) in 2011 to 11.4% (8/70) in 2017 was significant ($p = 0.0175$) (Fig. 2a). Interestingly, the only two *erm*(C)-positive isolates in 2011 belonged to CCs 5 and 8, whilst the eight *erm*(C)-positive isolates in 2017 were assigned to four CCs including CC5 ($n = 4$), CC45 ($n = 2$), CC8 ($n = 1$) and CC1 ($n = 1$) (Supplemental Table S1). Only 6.0% (15/252) of isolates exhibited multidrug resistance, defined as the presence of genes encoding resistance to three or more classes of antimicrobial agents/disinfectants, and this decreased from 10.7% (9/84) in 2006/2007 to 3.1% (3/98) and 4.2% (3/70) in 2011 and 2017, respectively, with the decrease from 2006/2007 to 2011 approaching statistical significance ($p = 0.0683$). This

Table 1 Demographic data for the patients in Irish hospitals with MSSA bloodstream infections during 6-month study periods in 2006/2007, 2011 and 2017

	Number of isolates (%)[a]		
	2006/2007	2011	2017
Male	54/84 (64%)	62/98 (63%)	44/70 (63%)
CO-MSSA	28/67 (42%)	48/90 (53%)	46/66 (70%)
HO-MSSA	39/67 (58%)	42/90 (47%)	20/66 (30%)
14-day all-cause mortality rate	7/72 (10%)	10/88 (11%)	5/54 (9%)
Age range			
0–9	11/84 (13%)	12/90 (13%)	4/70 (6%)
10–19	5/84 (6%)	2/90 (2%)	1/70 (1.5%)
20–29	5/84 (6%)	2/90 (2%)	1/70 (1.5%)
30–39	4/84 (5%)	9/90 (10%)	5/70 (7%)
40–49	6/84 (7%)	8/90 (9%)	6/70 (9%)
50–59	9/84 (11%)	9/90 (10%)	11/70 (16%)
60–69	16/84 (19%)	17/90 (19%)	10/70 (14%)
70–79	15/84 (18%)	10/90 (11%)	19/70 (27%)
80–89	13/84 (15%)	17/90 (19%)	12/70 (17%)
90+	0	4/90 (4%)	1/70 (1.5%)

[a] The denominator values reflect the number of isolates for which the relevant information was available. The total number of isolates investigated in 2006/2007, 2011 and 2017 was 84, 98 and 70, respectively

CO community onset, HO hospital onset

included combinations of genes encoding resistance to penicillin (100%, 15/15; *blaZ*), macrolides, lincosamides and streptogramin B compounds (60%, 9/15; *lnu*(A), *erm*(A) and/or *erm*(C)), aminoglycosides (53.3%, 8/15; *aacA-aphD*, *aadD* and/or *aphA3-sat*), quaternary ammonium compounds (46.7%, 7/15; *qacA* or *qacC*), fusidic acid (40%, 6/15; *fusB* or *fusC*), tetracycline (33.3%, 5/15; *tet*(K)) and mupirocin (13.3%, 2/15; *ileS2*).

Toxin genes The enterotoxin gene cluster (*egc*: *seg*, *sei*, *sem*, *sen*, *seo* and *seu*) was common but there was a significant decrease in the carriage rate from 72.6% (61/84) in 2006/2007 to 57.1% (56/98) in 2011 ($p = 0.0134$). Whilst the prevalence of *egc* increased again in 2017 to 70% (49/70), this was not significant ($p = 0.1068$) (Fig. 2b). The prevalence of *sec*/*sel* increased across all study periods (11.9% in 2006/2007 [10/84], 15.3% in 2011 [15/98] and 21.4% in 2017 [15/70]) but this was not significant (Fig. 2b). The prevalence of *tst* decreased across the study periods from 20.2% (17/84) to 16.3% (16/98) to 10% (7/70) in 2006/2007, 2011 and 2017, respectively, but this was not significant. The majority of *tst*-positive isolates (28/40) were assigned to CC30/ST34/42, and the prevalence of CC30 MSSA declined significantly during the study (Fig. 1a). Interestingly, the non-CC30/ST34/42 *tst*-positive isolates were only identified in 2006/2007 and 2011 and were assigned to CC1, CC5, CC6, CC7, CC8 and ST1093. The remaining toxin genes were identified in small numbers of isolates but the majority were identified during all three study periods, apart from the exfoliative and epidermolysis toxins *etB*, *etD/edinB* and *edinC* and the Panton-Valentine leukocidin (PVL) genes *lukF/S-PV*, none of which were identified in 2017 (Fig. 2b). Only two isolates were *lukF/S-PV* positive and were both identified in 2006/2007 and belonged to CC45-t362 and CC59-t216.

Typing markers, biofilm and adhesion genes Although variations in the prevalence of the *agr* types were detected across the study periods, these were not significant (Fig. 2c) and *agr* type I predominated, increasing from 45% in 2006/2007 (38/84) and 2011 (44/98) to 55.7% (39/70) in 2017. Capsule type 8 predominated (61.9%, 156/252), although a slight decrease in its prevalence and increase in capsule type 5 was detected across the study periods (Fig. 2c). Polysaccharide intercellular adhesin (PIA) encoded by the *ica* operon was detected in the majority of isolates (98.4%, 248/252) with no significant differences in prevalence identified (Fig. 2c).

Various combinations of the immune evasion complex (IEC) genes (*sea*, *sep*, *sak*, *chp* and *scn*) were also detected in the majority of isolates (92.1%, 232/252) in all study periods (94.1% in 2006/2007 [79/84], 90.8% in 2011[89/98], 91.4% in 2017 [64/70]) (Fig. 2c). The IEC-negative isolates belonged to CC5 (t179, t071, t002, t548), CC30 (t1827, t021, t012, t8304), ST34 (t166), CC1 (t127), CC22 (t2945), CC9 (t100) and CC8 (t008). Amongst the IEC-positive isolates, IEC type B (*sak*, *chp* and *scn*) predominated (51.2% in 2006/2007 [43/84], 35.7% in 2011 [35/98], 48.6% in 2017

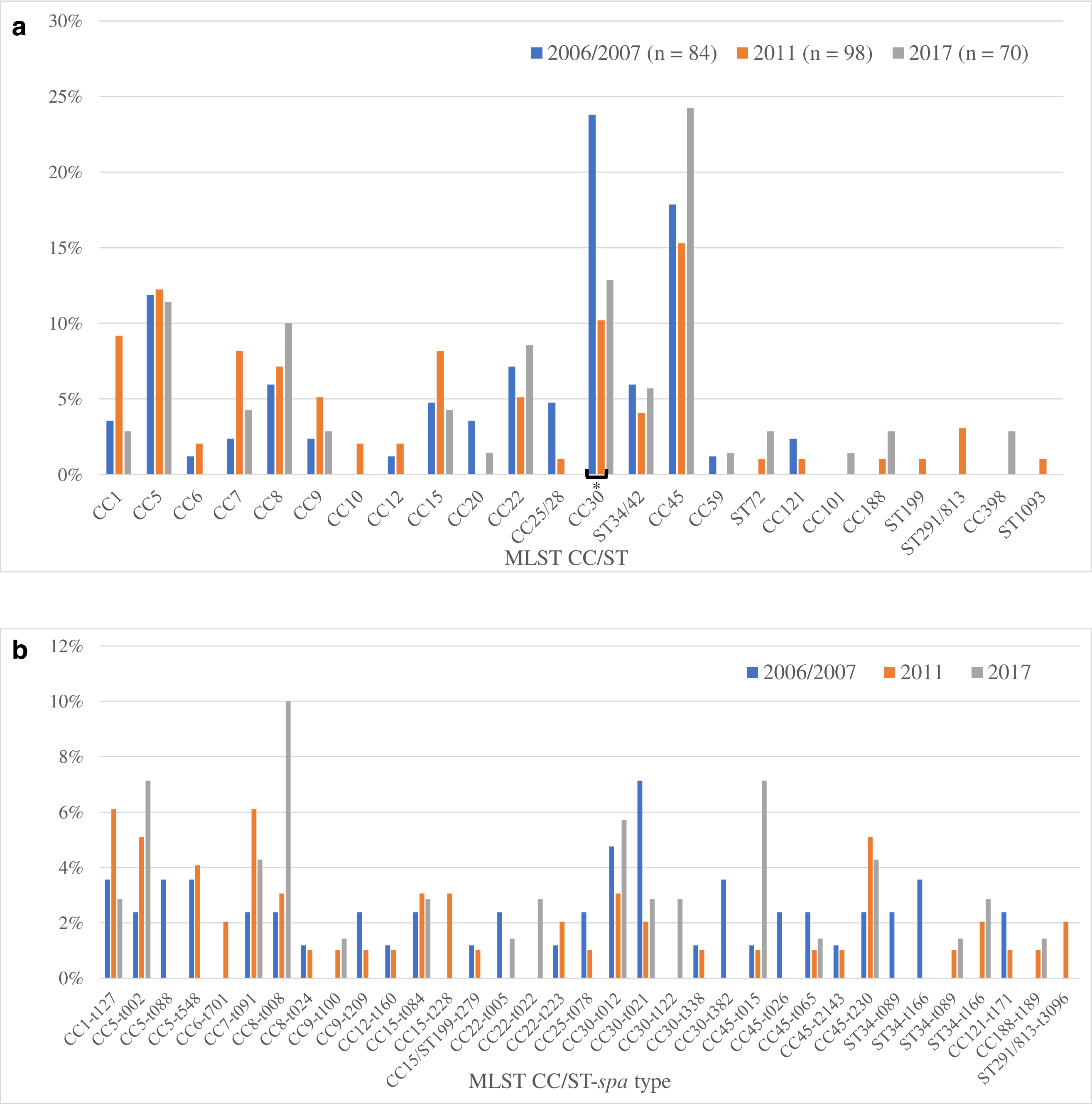
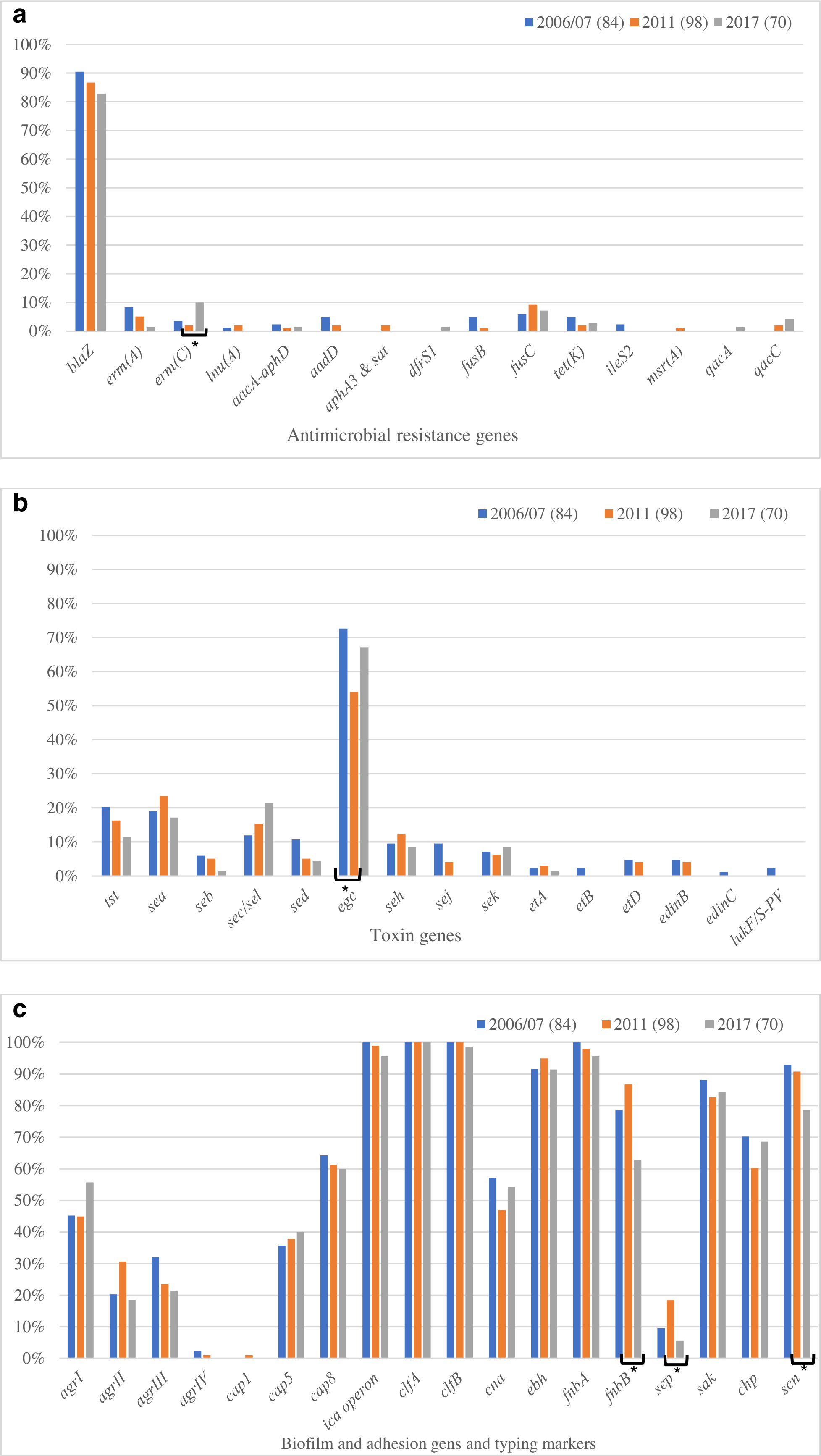


Fig. 1 Genotyping data for the MSSA isolates recovered from patients in Irish hospital during 6-month study periods in 2006/2007, 2011 and 2017. **a** Sequence types (STs) and/or clonal complexes (CCs) assigned by DNA microarray profiling to the 252 MSSA isolates. **b** MLST CC and *spa* types represented by ≥ 2 isolates identified amongst the 252 MSSA isolates. Changes in the percentage of isolates represented by each genotype between 2 years that are significant are labelled with an asterisk with the 2 years in question indicated with a small black horizontal and vertical lines

[34/70]) (Supplemental Table S2), but these differences were not significant. Amongst the individual IEC genes, the most notable changes were a significant decrease in *scn* from 90.8% (89/98) in 2011 to 78.6% (55/70) in 2017 ($p = 0.0424$) and *sep* from 18.4% (18/98) in 2011 to 5.7% (4/70) in 2017 ($p = 0.0197$). The *scn*-negative isolates were assigned to multiple of CCs/STs, and an increasing variety of *scn*-negative clones were identified in 2017 compared to the previous study periods (Supplemental Fig. S1(a)). The *sep*-negative isolates were assigned to an extensive range of CCs/STs (Supplemental Fig. S1(b)).

Most notable amongst the adhesion genes was the significant decrease ($p = 0.0008$) in the prevalence of the fibronectin binding protein gene *fnbB* from 86.7% (85/98) in 2011 to


a
2006/07 (84)
2011 (98)
2017 (70)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
blaZ
erm(A)
erm(C)
*
lnu(A)
aacA-aphD
aadD
aphA3 & sat
dfrS1
fusB
fusC
tet(K)
ileS2
msr(A)
qacA
qacC
Antimicrobial resistance genes
b
2006/07 (84)
2011 (98)
2017 (70)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
tst
sea
seb
sec/sel
sed
egc
*
seh
sej
sek
etA
etB
etD
edinB
edinC
lukF/S-PV
Toxin genes
c
2006/07 (84)
2011 (98)
2017 (70)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
agrI
agrII
agrIII
agrIV
cap1
cap5
cap8
ica operon
clfA
clfB
cna
ebh
fnbA
fnbB
*
sep
*
sak
chp
scn
*
Biofilm and adhesion gens and typing markers

◀ **Fig. 2** **a** Antimicrobial resistance genes, **b** toxin genes and **c** biofilm and adhesion genes and typing markers, identified following DNA microarray profiling of 252 MSSA isolates recovered from patients in Irish hospital with bloodstream infections during 6-month study periods in 2006/2007, 2011 and 2017. Changes in the percentage of isolates represented by each genotype between 2 years that are significant are labelled with an asterisks, with the 2 years in question indicated with a small black horizontal and vertical lines

64.3% (45/70) in 2017 (Fig. 2c). No specific clonal trends were identified although an increase in CC22, CC30 and CC45 *fnbB*-negative isolates was identified in 2017 (Supplemental Fig. S1(c)).

Discussion

The most interesting findings from the patient data in the present study were a significant increase and decrease in CO-MSSA and HO-MSSA BSIs, respectively, particularly between 2006/2007 and 2017, as well as a significant increase in the age of patients. Older age has been shown previously to increase the risk of *S. aureus* BSIs [27]. The increasing age of patients with MSSA BSIs may be due to the ageing population in Ireland. According to the Irish Central Statistics Office, 37.2% of the Irish population in 2016 was aged ≥ 45 years compared to 34.4% in 2011 and 32.9% in 2006, and between 2011 and 2016, there was a 19.1% increase in those > 65 years old [28]. Other factors such as socio-economic status, ethnicity and co-morbidities have also been shown to increase the risk of BSIs [27], but these were not investigated in the present study. A number of other studies have also reported an increase in age and/or incidence of CO-MSSA for patients with MSSA BSIs [12, 27]. This is alarming as it has been reported previously that CO-MSSA BSIs are more likely to be metastatic than HO-MSSA BSIs and have a worse 30-day outcome [29]. Although the latter was not reflected in the findings of the present study as no significant difference was detected in the mortality rate for CO- and HO-MSSA, it is important to note that in the present study a 14-day all-cause mortality rate was used due to study constraints in following up on post-discharge surveillance of patients, compared to 30 days in the previous study. The decreasing prevalence of HO-MSSA may be reflective of improvements in hospital infection, prevention and control procedures such as increased compliance with hand hygiene as reported for healthcare workers in Irish hospitals between 2011 and 2018 [30].

Despite the extensive genetic diversity detected amongst the MSSA BSI isolates investigated, almost 50% were assigned to just three MLST CCs (CCs 45, 30 and 5) that predominated, albeit in different proportions, during each study period. These were also the top 3 MLST CCs identified amongst MSSA in a recent study from patients with BSIs in Spanish hospitals between 2002 and 2017, were amongst the top four MLST-CCs from a study of MSSA from infection and colonisation between 1992 and 2011 in Portugal and from BSIs in a hospital in Minnesota, USA, in 2015 and were amongst the top six clones identified in the European *spa* typing studies in 2006 and 2011 [12, 13, 19–21]. In contrast, a Romanian study revealed a predominance of CC1, CC22 and CC45 MSSA, and studies from Africa and Asia revealed the predominance of different CCs amongst MSSA from BSIs, indicating geographical variation in the predominant MSSA clonal types [18, 22–24]. Interestingly, CC22-MSSA isolates identified in the present study could potentially be precursors to the predominant MRSA clone in Irish hospitals, ST22-MRSA-IV, with several of the *spa* types identified here, e.g. t022, t032, t2945 and t005, being found previously to be common amongst nosocomial ST22-MRSA-IV [16]. However, it is also possible that they represent MRSA strains that have lost SCC*mec*. A Portuguese study previously identified MSSA that were potential precursors to CA-MRSA [21]. Whole-genome sequencing could be used to determine the relatedness, between these MRSA and MSSA isolates exhibiting the same *spa* type, although this was beyond the scope of the present study. Also worth noting is the lack of IEC genes detected amongst 7.9% of isolates (20/252), suggestive of a possible animal origin for some isolates, which has been reported previously for some of the genotypes identified here [31–33]. However, the present study did not collate any data related to potential zoonotic sources of infections and the lack of IEC genes more than likely reflects evolutionary origin rather than direct transmission between animals and humans.

There was some evidence from the present study to suggest that the increasing prevalence of MSSA BSIs in Ireland could be linked to the changing molecular epidemiology of MSSA, including a small increase in the MLST CC/ST per isolate ratio across the three study periods and variation in the prevalence of some of the more common clones identified. In relation to the latter, the overall prevalence of CC30, in particular CC30-t021, which was found to be more commonly associated with HO-MSSA, declined significantly between 2006/2007 and 2011 and CC45, most notably CC45-t015, increased, as did CC8-t008, with both of these clones being more commonly associated with CO-MSSA. In fact, 5/7 most common *spa* types identified in the present study were more common amongst CO- compared to HO-MSSA, suggesting a potential link between specific clones and CO-MSSA BSIs. Although a previous study of MSSA BSIs in Spanish hospitals found CC5 to be more common amongst hospital-associated rather than community-associated (CA) *S. aureus*, that study included both MRSA and MSSA and no such association was identified for CC5 MSSA in the present study [20].

The increasing prevalence of CO-MSSA was coupled with an overall decrease in the prevalence of multidrug resistance and some virulence genes, amongst the isolates. The decrease in antimicrobial resistance could be reflective of the community source of many of these MSSA isolates, where the selective pressure for the development of multidrug resistance is lower than in a hospital setting [34]. In contrast, there was a significant increase in the prevalence of the macrolide, lincosamide and streptogramin B resistance gene *erm*(C) between 2011 and 2017, and the increasing diversity of clones harbouring this gene suggests that horizontal transfer of this predominantly plasmid-located gene may have occurred, although further studies are required to confirm this. An overall reduction in the number of resistance and virulence genes could potentially result in increased fitness due to the carriage and expression of less genes in these isolates [35, 36]. The decrease in the prevalence of virulence genes was predominantly associated with the superantigen/enterotoxin gene cluster *egc*, the lysogenic phage located IEC genes *scn* and *sep* encoding a staphylococcal complement inhibitor and a staphylococcal enterotoxin, respectively, the superantigen gene encoding the toxic shock toxin (*tst*) and the microbial surface component recognising adhesive matrix molecules gene *fnbB* encoding fibronectin binding protein. Yu et al. [24] identified less antimicrobial resistance and virulence genes, in particular superantigen and *pvl* genes, amongst MSSA compared to MRSA from BSIs between 2004 and 2010. The *pvl* gene was rare amongst isolates in the present study, only being detected in two isolates (0.8%). Previous studies have also reported a low prevalence of *pvl* amongst *S. aureus* from BSIs, and it is more commonly associated with *S. aureus* from skin and soft tissue infections [37–39].

The results of this study suggest that the increasing prevalence of MSSA BSIs is the result of a combination of changes in both patient- and pathogen-specific factors including an increase in CO-MSSA, particularly CC45 CO-MSSA, a decrease in HO-MSSA, particularly CC30 HO-MSSA, an ageing population and a reduction in the prevalence of some virulence genes and overall multidrug resistance. This highlights the need for increased awareness of the risk of MSSA BSIs, in particular CO-MSSA, in a vulnerable ageing population and targeted infection prevention and control procedures are required to reduce the prevalence of these serious and life-threatening infections. Further studies are required to investigate the significance of the reduced prevalence of virulence genes amongst the MSSA isolates and to further investigate the potential link between specific MSSA clones and CO/HO-BSIs.

Acknowledgments We thank the hospitals for referring their isolates to the NMRSARL and the staff, past and present, of the NMRSARL, for technical assistance.

Funding This work was supported by the Irish National MRSA Reference Laboratory and the Microbiology Research Unit, Dublin Dental University Hospital.

Compliance with ethical standards

Ethical approval and informed consent were not required for this study as isolates were collected as part of routine patient screening and no identifiable patient data was accessed for the study.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Goto M, Al-Hasan MN (2013) Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect 19:501–509. https://doi.org/10.1111/1469-0691.12195
2. European Centre for Disease Prevention and Control (ECDC) (2017) Surveillance of antimicrobial resistance in Europe 2016. https://ecdc.europa.eu/en/publications-data/antimicrobial-resistance-surveillance-europe-2016. Accessed 26 Nov 2018
3. Laupland KB (2013) Incidence of bloodstream infection: a review of population-based studies. Clin Microbiol Infect 19:492–500. https://doi.org/10.1111/1469-0691.12144
4. de Kraker MEA, Jarlier V, Monen JCM, Heuer OE, van de Sande N, Grundmann H (2014) The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. Clin Microbiol Infect 19:860–868. https://doi.org/10.1111/1469-0691.12028
5. Chong YP, Park SJ, Kim HS, Kim ES, Kim MN, Park KH, Kim SH, Lee SO, Choi SH, Jeong JY, Woo JH, Kim YS (2013) Persistent *Staphylococcus aureus* bacteremia. Medicine 92:98–108. https://doi.org/10.1097/MD.0b013e318289ff1e
6. Kaasch AJ, Barlow G, Edgeworth JD, Fowler VG, Hellmich M, Hopkins S, Kern WV, Llewelyn MJ, Rieg S, Rodriguez-Bano J, Scarborough M, Seifert H, Soriano A, Tilley R, Torok ME, Weiβ V, Wilson PR, Thwaites GE (2014) *Staphylococcus aureus* bloodstream infection: a pooled analysis of five prospective, observational studies. J Infect 68(3):242–251. https://doi.org/10.1016/j.jinf.2013.10.015
7. Seas C, Garcia C, Salles MJ, Labarca J, Luna C, Alvarez-Moreno C, Mejía-Villatoro C, Zurita J, Guzmán-Blanco M, Rodríguez-Noriega E, Reyes J, Arias CA, Carcamo C, Gotuzzo E (2017) *Staphylococcus aureus* bloodstream infections in Latin America: results of a multinational prospective cohort study. J Antimicrob Chemother 73:212–222. https://doi.org/10.1093/jac/dkx350
8. Harbarth S, Rutschmann O, Sudre P, Pittet D (1998) Impact of methicillin resistance on the outcome of patients with bacteremia caused by *Staphylococcus aureus*. Arch Intern Med 158:182–189
9. Tom S, Galbraith JC, Valiquette L, Jacobsson G, Collignon P, Schøheyder HC, Søgaard M, Kennedy KJ, Knudsen JD, Østergaard C, Lyytikainen O, Laupland KB (2014) Case fatality ratio and mortality rate trends of community-onset *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect 20:O630–O632. https://doi.org/10.1111/1469-0691.12564
10. European Antimicrobial Resistance Surveillance Network (EARS-Net) (2018) Irish Annual Report on Antimicrobial Resistance 2016. http://www.hpsc.ie/a-z/microbiologyantimicrobialresistance/

europeanantimicrobialresistancesurveillancesystemearss/ears-netdataandreports/annualreports/. Accessed 26 Nov 2018

11. Wilson J, Elgohari S, Livermore DM, Cookson B, Johnson A, Lamagni T, Chronias A, Sheridan E (2014) Trends among pathogens reported as causing bacteraemia in England, 2004–2008. Clin Microbiol Infect 17:451–458. https://doi.org/10.1111/j.1469-0691.2010.03262.x
12. Grundmann H, Schouls LM, Aanensen DM, Pluister GN, Tami A, Chlebowicz M, Glasner C, Sabat AJ, Weist K, Heuer O, Friedrich AW, ESCMID Study Group on Moleculae Epidemiological Markers; European Staphylococcal Reference Laboratory Working Group (2014) The dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: results of a second structured survey. Euro Surveill 19(49). https://doi.org/10.2807/1560-7917.es2014.19.49.20987.
13. Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW, European Staphylococcal Reference Laboratory Working Group (2010) Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med 7:e1000215–e1000215. https://doi.org/10.1371/journal.pmed.100021
14. Holden MTG, Hsu L-Y, Kurt K, Weinert LA, Mather AE, Harris SR, Strommenger B, Layer F, Witte W, de Lencastre H, Skov R, Westh H, Zemlickova H, Coombs G, Kearns AM, Castillo-Ramirez S, Feil EJ, Hudson LO, Enright MC, Balloux F, Aanensen DM, Spratt BG, Fitzgerald JR, Achtman M, Bentley SD, Nubel U (2013) A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. Genome Res 23:653–664. https://doi.org/10.1101/gr.147710.112
15. Irish National MRSA Reference Laboratory. Annual report 2015. http://www.stjames.ie/Departments/DepartmentsA-Z/N/NationalMRSAReferenceLaboratory/DepartmentinDepth/AnnRpt2015.pdf. Accessed 26 Nov 2018
16. Shore AC, Rossney AS, Kinnevey PM, Brennan OM, Creamer E, Sherlock O, Dolan A, Cunney R, Sullivan DJ, Goering RV, Humphreys H, Coleman DC (2010) Enhanced discrimination of highly clonal ST22-methicillin-resistant *Staphylococcus aureus* IV isolates achieved by combining *spa*, *dru*, and pulsed-field gel electrophoresis typing data. J Clin Microbiol 48(5):1839–1852. https://doi.org/10.1128/JCM.02155-09
17. Wehrhahn MC, Robinson JO, Pascoe EM, Coombs GW, Pearson JC, O'Brien FG, Tan HL, New D, Salvaris P, Salvaris R, Murray RJ (2012) Illness severity in community-onset invasive *Staphylococcus aureus* infection and the presence of virulence genes. J Infect Dis 205:1840–1848. https://doi.org/10.1093/infdis/jis279
18. Dekker D, Wolters M, Mertens E, Boahen KG, Krumkamp R, Eibach D, Schwarz NG, Adu-Sarkodie Y, Rohde H, Christner M, Marks F, Sarpong N, May J (2016) Antibiotic resistance and clonal diversity of invasive *Staphylococcus aureus* in the rural Ashanti Region, Ghana. BMC Infect Dis 16:1–6. https://doi.org/10.1186/s12879-016-2048-3
19. Park K-H, Greenwood-Quaintance KE, Uhl JR, Cunningham SA, Chia N, Jeraldo PR, Sampathkumar P, Nelson H, Patel R (2017) Molecular epidemiology of *Staphylococcus aureus* bacteremia in a single large Minnesota medical center in 2015 as assessed using MLST, core genome MLST and *spa* typing. PLoS One 12:e0179003–e0179012. https://doi.org/10.1371/journal.pone.0179003
20. Pérez-Montarelo D, Viedma E, Larrosa N, Gómez-González C, Ruiz de Gopegui E, Muñoz-Gallego I, San Juan R, Fernández-Hidalgo N, Almirante B, Chaves F (2018) Molecular epidemiology of *Staphylococcus aureus* bacteremia: association of molecular factors with the source of infection. Front Microbiol 9:2210. https://doi.org/10.3389/fmicb.2018.02210
21. Tavares A, Faria NA, Lencastre H, Miragaia M (2013) Population structure of methicillin-susceptible *Staphylococcus aureus* (MSSA) in Portugal over a 19-year period (1992–2011). Eur J Clin Microbiol Infect Dis 33:423–432. https://doi.org/10.1007/s10096-013-1972-z
22. Vandendriessche S, De Boeck H, Deplano A, Phoba MF, Lunguya O, Falay D, Dauly N, Verhaegen J, Denis O, Jacobs J (2017) Characterisation of *Staphylococcus aureus* isolates from bloodstream infections, Democratic Republic of the Congo. Eur J Clin Microbiol Infect Dis 36:1–9. https://doi.org/10.1007/s10096-017-2904-0
23. Monecke S, Müller E, Dorneanu OS, Vremeră T, Ehricht R (2014) Molecular typing of MRSA and of clinical *Staphylococcus aureus* isolates from Iaşi, Romania. PLoS One 9:e97833–e97839. https://doi.org/10.1371/journal.pone.0097833
24. Yu F, Li T, Huang X, Xie J, Xu Y, Tu J, Qin Z, Parsons C, Wang J, Hu L, Wang L (2012) Virulence gene profiling and molecular characterization of hospital-acquired *Staphylococcus aureus* isolates associated with bloodstream infection. Diagn Microbiol Infect Dis 74:363–368. https://doi.org/10.1016/j.diagmicrobio.2012.08.015
25. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2018) Breakpoint tables for interpretation of MICs and zone diameters 2016. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf
26. Monecke S, Slickers P, Ehricht R (2008) Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol Med Microbiol 53:237–251. https://doi.org/10.1111/j.1574-695X.2008.00426.x
27. Laupland KB, Lyytikäinen OL, Søgaard M, Kennedy KJ, Knudsen JD, Ostergaard C, Galbraith JC, Valiquette L, Jacobsson G, Collignon P, Schønheyder HC, International Bacteremia Surveillance Collaborative (2014) The changing epidemiology of *Staphylococcus aureus* bloodstream infection: a multinational population-based surveillance study. Clin Microbiol Infect 19:465–471. https://doi.org/10.1111/j.1469-0691.2012.03903.x
28. Central Statistics Office (CSO) Ireland (2018) Census 2016 Profile 3—an age profile of Ireland. https://www.cso.ie/en/csolatestnews/presspages/2017/census2016profile3-anageprofileofireland/. Accessed 26 Nov 2018
29. Melzer M, Welch C (2013) Thirty-day mortality in UK patients with community-onset and hospital-acquired meticillin-susceptible *Staphylococcus aureus* bacteraemia. J Hosp Infect 84:143–150. https://doi.org/10.1016/j.jhin.2012.12.013
30. Health Protection Surveillance Centre (2019) Hand hygiene compliance. http://www.hpsc.ie/a-z/microbiologyantimicrobialresistance/europeansurveillanceofantimicrobialconsumptionesac/PublicMicroB/HHA/HHA_Current.pdf. Accessed 26 Nov 2018
31. BystroÅ JA, Podkowik M, Piasecki T, Wieliczko A, Molenda J, Bania J (2010) Genotypes and enterotoxin gene content of *S. aureus* isolates from poultry. Vet Microbiol 144:498–501. https://doi.org/10.1016/j.vetmic.2010.01.029
32. Mroczkowska A, Żmudzki J, Marszałek N, Orczykowska-Kotyna M, Komorowska I, Nowak A, Grzesiak A, Czyżewska-Dors E, Dors A, Pejsak Z, Hryniewicz W, Wyszomirski T, Empel J (2017) Livestock-associated *Staphylococcus aureus* on Polish pig farms. PLoS One 12:e0170745–e0170718. https://doi.org/10.1371/journal.pone.0170745
33. Alba P, Feltrin F, Cordaro G, Porrero MC, Kraushaar B, Argudín MA, Nykäsenoja S, Monaco M, Stegger M, Aarestrup FM, Butaye P, Franco A, Battisti A (2015) Livestock-associated methicillin resistant and methicillin susceptible *Staphylococcus aureus* sequence type (CC)1 in European farmed animals: high genetic relatedness of isolates from Italian cattle herds and humans. PLoS One 10:

e0137143–e0137110. https://doi.org/10.1371/journal.pone.0137143

34. Health Service Executive and Health Protection Surveillance Centre (2018) Annual epidemiological report on antimicrobial consumption in Ireland, 2017. http://www.hpsc.ie/a-z/microbiologyantimicrobialresistance/europeansurveillanceofantimicrobialconsumptionesac/surveillancereports/AER_AntimicrobialConsumption_2017.pdf. Accessed 26 Nov 2018
35. Challagundla L, Reyes J, Rafiqullah I, Sordelli DO, Echaniz-Aviles G, Velazquez-Meza ME, Castillo-Ramírez S, Fittipaldi N, Feldgarden M, Chapman SB, Calderwood MS, Carvajal LP, Rincon S, Hanson B, Planet PJ, Arias CA, Diaz L, Robinson DA Phylogenomic classification and the evolution of clonal complex 5 methicillin-resistant *Staphylococcus aureus* in the Western Hemisphere. Front Microbiol 9:2197–2114. https://doi.org/10.3389/fmicb.2018.01901
36. Yokoyama M, Stevens E, Laabei M, Bacon L, Heesom K, Bayliss S, Ooi N, O'Neill AJ, Murray E, Williams P, Lubben A, Reeksting S, Meric G, Pascoe B, Sheppard SK, Recker M, Hurst LD, Massey RC (2018) Epistasis analysis uncovers hidden antibiotic resistance-associated fitness costs hampering the evolution of MRSA. Genome Biol 19(1):94. https://doi.org/10.1186/s13059-018-1469-2
37. Ellington MJ, Hope R, Ganner M, Ganner M, East C, Brick G, Kearns AM (2007) Is Panton–Valentine leucocidin associated with the pathogenesis of *Staphylococcus aureus* bacteraemia in the UK? J Antimicrob Chemother 60(2):402–405. https://doi.org/10.1093/jac/dkm206
38. Saeed K, Gould I, Esposito S, Ahmad-Saeed N, Ahmed SS, Alp E, Bal AM, Bassetti M, Bonnet E, Chan M, Coombs G, Dancer SJ, David MZ, De Simone G, Dryden M, Guardabassi L, Hanitsch LG, Hijazi K, Krüger R, Lee A, Leistner R, Pagliano P, Righi E, Schneider-Burrus S, Skov RL, Tattevin P, Van Wamel W, Vos MC, Voss A, International Society of Chemotherapy (2018) Panton–Valentine leukocidin-positive *Staphylococcus aureus*: a position statement from the International Society of Chemotherapy. Int J Antimicrob Agents 51(1):16–25. https://doi.org/10.1016/j.ijantimicag.2017.11.002
39. Statens Serum Institut and National Food Institute, Technical University of Denmark (2018) DANMAP 2017. https://www.danmap.org/-/media/arkiv/projekt-sites/danmap/danmap-reports/danmap-2017/danmap2017.pdf?la=en. Accessed 26 Nov 2018