Universal versus targeted chlorhexidine and mupirocin decolonisation and clinical and molecular epidemiology of *Staphylococcus epidermidis* bloodstream infections in patients in intensive care in Scotland, UK: a controlled time-series and longitudinal genotypic study

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Summary

Background There are concerns that biocide skin and mucous membrane decolonisation, which is widely used to prevent health-care-associated infections in intensive care units (ICUs), might select for multidrug-resistant pathogens. We aimed to evaluate the effects of de-escalating from universal to targeted skin and nasal decolonisation on *Staphylococcus epidermidis* bloodstream infections (SE-BSI).

Methods We did a retrospective, before-after-control-impact time-series analysis and longitudinal genotypic study in two ICUs with divergent decolonisation practice in tertiary care hospitals of adjacent health boards in Scotland, UK. Participants were aged at least 16 years and admitted between July 1, 2009, and Feb 28, 2022. There were no exclusion criteria for the study. In ICU one (intervention site) universal decolonisation in all admissions was de-escalated to targeted decolonisation of meticillin-resistant *Staphylococcus aureus* (MRSA) carriers on Feb 1, 2019, while in ICU two (control site) targeted decolonisation was applied throughout. We collected bloodstream infection data from all causes, including clinically significant SE-BSI. Antimicrobial susceptibility testing was used to define meticillin-resistant *S epidermidis* (MRSE) and chlorhexidine susceptibility. We used multilocus sequence typing to identify sequence types from archived SE-BSI isolates. Whole-genome sequencing was applied to a sample from ICU one. The primary outcomes were incidence densities of all bloodstream infections, SE-BSI, and meticillin-resistant *S epidermidis* bloodstream infections (MRSE-BSI), and the percentage probability that SE-BSI were MRSE-BSI. The effects of de-escalation on primary outcomes were estimated by differences between the intervention and control sites, before and after de-escalation, using a before-after-control-impact time-series design. Secondary outcomes included the proportion of multidrug resistant sequence types, carriage of mobile genetic elements and genes for multidrug resistance and biofilm production.

Findings Between July 1, 2009, and Feb 28, 2022, *S epidermidis* was identified in 334 (45%) of 735 bloodstream infections in ICU one, of which 197 occurred before the de-escalation intervention in Feb 1, 2019, and *S epidermidis* was identified in 167 (60%) of 278 bloodstream infections in ICU two. There was no increase in all bloodstream infection incidence coinciding with de-escalation in ICU one, whereas MRSE-BSI incidence declined significantly from 10·4 cases per 1000 occupied bed days (OBDs; 95% credible interval [CrI] 7·2–15·4) to 4·3 cases per 1000 OBDs (2·5–6·7), as did the percentage probability of MRSE (from 89·2%, 95% CrI 77·8–96·5 to 56·7%, 34·3–77·5%). No significant changes in the primary outcomes were seen in ICU two. MRSE-BSI incidence density was positively associated with chlorhexidine use, but not mupirocin use. De-escalation was associated with a reduced proportion of SE-BSI due to multidrug-resistant sequence types and reduced carriage of mobile genetic elements and genes for multidrug resistance and biofilm production, as observed by multi-locus sequence typing and whole genome sequencing.

Interpretation In ICU settings with low MRSA incidence, the benefits of universal decolonisation should be balanced against the risks of selecting MRSE sequence types adapted for invasive and device-associated infection.

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Introduction

Staphylococcus epidermidis is a leading cause of intensive care unit (ICU)-acquired bloodstream infections and infections associated with medical devices.[1] The pathogenic importance of this ubiquitous skin and mucous membrane coloniser has been documented, particularly in relation to predisposing host factors, as has its role as a reservoir of multidrug-resistance genes.[2] The global population structure of hospital-associated *S epidermidis* belongs to a single genetic cluster that is strongly associated with multidrug resistance and pathogenic phenotypes such as biofilm formation.[3,4] The global dissemination of this cluster has underpinned concerns about horizontal transfer of multidrug-resistance gene islands to other species, particularly in the context of selective pressure by intensive use of topical biocides to prevent hospital-associated infections in specific clinical settings.[5,6] Chlorhexidine is one such biocide and is widely used for skin and mucous membrane decontamination in ICUs, pre-operative skin disinfection, and impregnation of medical devices.[7] Chlorhexidine has attracted attention in view of early outbreaks of chlorhexidine-tolerant meticillin-resistant *Staphylococcus aureus* (MRSA) clones.[8] This phenotype is strongly associated with the *qacA/B* gene, notwithstanding other implicated evasion mechanisms.[9] The co-location of genes associated with reduced susceptibility to chlorhexidine and mupirocin and antibiotic resistance genes on variants of known MRSA plasmids has led to a hypothesis of selective environmental pressure by biocides contributing to the increasing prevalence of multidrug-resistant lineages of *S epidermidis*.[5,10–12] We previously studied a global collection of 231 isolates of *S epidermidis* and found significant enrichment of genes associated with biocide tolerance in the three hospital-adapted multidrug-resistant lineages, namely ST2, ST2-BPH0662, and ST23.[5] Nonetheless, to our knowledge, there is no direct evidence of a relationship between

Research in context

Evidence before this study

To capture studies linking concurrent chlorhexidine-based and mupirocin-based decolonisation to reduced biocide susceptibility and antibiotic resistance in clinical isolates of staphylococcal species, we searched Medline between Jan 1, 1995, and Jan 23, 2025, with no language restrictions, using the terms (chlorhexidine or CHG or biocide) AND (mupirocin) AND (MRSA or staphylococcus aureus or CoNS or coagulase-negative staphylococcus or Staphylococcus epidermidis) AND (non susceptib* or antibiotic resist* or MDR or multidrug resist* or antimicrobial resist* or cross-resistance or cross-selection or qacA* or biocide resist*). Descriptive studies and cross-sectional comparisons were excluded. A cluster-randomied multicentre trial (REDUCE-MRSA, NCT00980980), a single-site randomised controlled trial in a trauma population (NCT01820455), and another randomised controlled trial in a paediatric population reported rare or no reduced phenotypic or genetic susceptibility to chlorhexidine in patients decolonised with chlorhexidine and mupirocin. In the paediatric study, decolonisation was less effective in mupirocin-resistant *Staphylococcus aureus* carriers, but REDUCE-MRSA reported no significant effects on mupirocin resistance rates, notwithstanding broad confidence intervals. Follow-up periods for these studies were 12–22 months. Two retrospective cohort studies reported low prevalence of mupirocin resistance or reduced susceptibility to chlorhexidine in targeted decolonisation of *S aureus* carriers. Two before-after studies reported associations between decolonisation and increased *S aureus* low-level mupirocin resistance, with one universal decolonisation study showing high-level mupirocin resistance increase in coagulase-negative staphylococci after treatment. A nested case-control study of targeted decolonisation suggested that low-level mupirocin and genes for reduced susceptibility to chlorhexidine increase meticillin-resistant *S aureus* persistence. Evidence of the effects of biocide exposure on multidrug-resistant staphylococci is scarce, with short follow-up periods in experimental studies and high risks of bias in observational studies.

Added value of this study

To our knowledge, this study provides the first direct evidence of a relationship between universal decolonisation, chlorhexidine exposure, and infection with pathogenic meticillin-resistant (multidrug-resistant) *Staphylococcus epidermidis* (MRSE). In controlled time-series analyses, we estimated that de-escalation from universal to targeted decolonisation substantially reduced rates of MRSE bloodstream infection but did not modify the overall incidence of bacterial and fungal bloodstream infections. Risks of bias due to confounders were minimised by adjustment for expected temporal behaviour based on pre-intervention data and contemporary changes in the control population. We found that de-escalation in the intervention site reduced multidrug-resistant *S epidermidis* sequence types, particularly ST2. Consistent with a hypothesis of co-selection, isolates with reduced susceptibility to chlorhexidine were more likely to show phenotypic resistance to multiple antibiotics and carry a biofilm-associated operon and mobile genetic elements associated with multidrug resistance and increased fitness.

Implications of all the available evidence

Our findings add to early evidence indicating that intensive use of biocides in health care co-selects biocide-tolerant and antibiotic-resistant *S epidermidis*. Our results show that universal decolonisation has contributed to the expansion of hospital-adapted multidrug-resistant lineages of *S epidermidis*. Regulation of biocide use could prevent unintended harms. Alongside development of alternative treatment strategies, such as targeted decolonisation and microbiota repopulation strategies, future studies should investigate the effects of universal decolonisation on other multidrug-resistant organisms.

biocide exposure and expansion of multidrug-resistant clones of *S epidermidis*.

Skin and nasal decolonisation in ICUs using chlorhexidine and mupirocin, respectively, has been delivered either universally to all admissions or targeted to high-risk MRSA admissions. Despite similar trends in MRSA incidence, practices in Scottish ICUs differed over time, providing a unique opportunity for a natural experiment. In this study, we used 12 years of data and archived isolates from two ICUs with divergent decolonisation practices to assess the effects of sustained use of universal versus targeted decolonisation and de-escalation from universal to targeted decolonisation on the profile of *S epidermidis* bloodstream infections.

Methods

Study design and participants

We undertook a retrospective controlled time-series and longitudinal genotypic study, using clinical auditing and bloodstream infection data and archived clinically significant *S epidermidis* bloodstream infection (SE-BSI) isolates from two ICUs in Scotland. The study sites were two medical-surgical ICUs in tertiary care hospitals of adjacent Health Boards (NHS Grampian and NHS Tayside) with divergent practices for skin and nasal decolonisation. In ICU one (intervention site), universal decolonisation was continuously implemented between May 1, 2001, and Jan 31, 2019, with de-escalation to targeted decolonisation based on MRSA admission screening from Feb 1, 2019. In ICU two (control site), targeted decolonisation was consistently applied throughout. The two ICUs had similar patient populations and baseline epidemiology in terms of MRSA incidence and total bloodstream infections. The median number of beds was 16 (ICU one) and eight (ICU two). Nearly all emergency admissions in each health board were from residents of the same health board.[13]

Study patients were aged 16 years and older and admitted to the two ICUs between Jul 1, 2009, and Jan 31, 2022; there were no exclusion criteria. The study period followed intensive national interventions to curb health-care-associated infections and was characterised by low MRSA incidence and tightly regulated antibiotic prescribing. Within each nursing shift, the nurse-to-patient ratio was 1:1 throughout the study.

Patients were anonymised and de-linked from personal data. Study site characteristics were derived from local anonymised data and aggregated data from national ICU audits. No patient-level data were used. Ethics review was not required, as confirmed by the North of Scotland Research Ethics Committee.

Procedures

Across all study months and sites, all admissions were screened for MRSA carriage within 24 h through combined clinical risk assessment (high risk if any of the following: previously MRSA positive; admission from care home or other hospital; wound or ulcer or invasive device present) and swabbing (from nose and perineum, or throat if perineal swabbing declined and, where applicable, swabs from wounds, lines, or sputum). All admitted patients were swabbed as per the national MRSA screening protocol.[14] High-risk or MRSA-positive patients were isolated and barrier-nursed with twice daily chlorine disinfection of their environment. Under universal decolonisation, all patients received 5 days of assisted washing with chlorhexidine gluconate 4% (appendix p 13) and three-times daily mupirocin 2% ointment to both nostrils (or four-times daily chlorhexidine 0·1% and neomycin 0·5% cream, if mupirocin resistant). Both skin and nasal decolonisation procedures were done by trained nursing staff. Individuals who were MRSA-positive on repeat screening 48 h after initial decolonisation received repeated decolonisation. In the event of earlier discharge, decolonisation was carried forward in the receiving ward for completion of the 5-day decolonisation regimen. Under targeted decolonisation, only MRSA carriers underwent decolonisation as described. Positive MRSA admission screens per 1000 occupied bed-days (OBDs) were obtained for both ICUs.

See Online for appendix

Antimicrobial consumption in ICU one was measured from hospital pharmacy monthly prescribing data. Both preparations were exclusively used for the 5-day skin and nasal decolonisation treatment.

Blood cultures were drawn from central or peripheral lines (or both) using the BD Vacutainer system (Becton Dickenson; Wokingham, UK) in aerobic and anaerobic bottles (BD BACTEC; Beckton Dickenson) and incubated at 37°C for 5 days before speciation. *S epidermidis* isolates were identified from blood cultures by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Ultraflex MALDI TOF/TOF Mass Spectrometer; Bruker Daltonics; Billerica, MA, USA). Spectra were acquired on flexControl version 3.4 software and analysed with MALDI Biotyper version 3.1. Susceptibilities to thirteen antibiotics were available from both sites (penicillin, flucloxacillin, ciprofloxacin, clindamycin, erythromycin, trimethoprim, gentamicin, mupirocin, fusidic acid, rifampicin, linezolid, nitrofurantoin, and vancomycin). There were no missing susceptibility data for these thirteen antibiotics. Since resistances to linezolid (n=3), nitrofurantoin (n=2), and vancomycin (n=1) were sporadic, and in the absence of a consensus definition, multidrug-resistant *S epidermidis* was defined by resistance to three or more of the other ten antibiotics. We used meticillin-resistant *S epidermidis* (MRSE) as a surrogate for multidrug resistance, as all MRSE isolates (as judged by oxacillin resistance available from ICU one for the whole study period) were resistant to at least three antibiotics. Antibiotic susceptibilities were from contemporary testing data obtained using a Vitek 2 AST card for Staphylococci (bioMérieux; Basingstoke, UK) over the study period, and chlorhexidine susceptibilities of archived isolates were batch tested using the agar dilution method (appendix p 2). European Committee on Antimicrobial Susceptibility

Testing (versions 1.0–12.0 for antibiotics, version 12.0 for chlorhexidine) recommendations were used for minimum inhibitory concentration (MIC) determination.

Multilocus sequence typing (MLST) was used to identify *S epidermidis* sequence types (STs) of available archived isolates from ICU one between Jul 1, 2009, and Jan 31, 2022, and from ICU two between Dec 1, 2016, and Jan 31, 2021. Genomic DNA was extracted using the PureLink Microbiome DNA Purification Kit (appendix p 2; Thermo Fisher Scientific; Paisley, UK). MLST was done on seven previously described highly discriminating genes, the allelic variations of which were analysed with the PubMLST platform to determine the ST.[15] PCR annealing temperatures were modified as follows: 60°C for *arc/aroE,* 52°C for *gtr/mutS/tpi/yqiL,* and 49°C for *pyrR.* Screening for the *mecA* and *qacA/B* gene was done with previously described primers.[4,9]

For the **PubMLST platform** see pubmlst.org

The genomes of a convenience sample of typed ICU one isolates (168 [67%] of 250 total ICU one isolates) were paired-end sequenced (350 base pairs) on a NovaSeq6000 platform (Illumina; San Diego, CA, USA) to identify genetic correlates of antimicrobial resistance (appendix p 2). The National Center for Biotechnology Information GenBank Bioproject identifiers for the genomes are PRJNA982045 and PRJNA574294. Accession numbers for each genome assembly are listed in the appendix (pp 14–15).

Phylogenetic relatedness was inferred from maximum likelihood analysis of the core genomes (1905 core genes out of 8121 genes per genome) using RAxML version 8.2.12 software with and without 362 already published genomes of pathogenic isolates from GenBank (National Institutes of Health).[10,16] Relatedness of ST2 isolates was investigated by calculation of core single nucleotide polymorphism (SNP) distances using snp-dists version 0.8.2. Details of genome assembly, quality control and analysis of SNP variation, phylogenetic relatedness, and carriage of antimicrobial resistance genes are reported in the appendix (pp 2–3).

Outcomes

The primary outcomes were incidence densities (cases per 1000 occupied bed days [OBDs]) of all bloodstream infections (*Enterococcus spp, S aureus, Klebsiella spp, Acinetobacter spp, Pseudomonas aeruginosa, Enterobacter spp, Escherichia coli*, and *Candida spp*); clinically significant SE-BSI and MRSE bloodstream infections (MRSE-BSI); and the percentage probability that SE-BSI were meticillin resistant (prMRSE-BSI). We also explored the relationship between MRSE-BSI incidence densities and volumes of chlorhexidine solution and mupirocin ointment prescribed. Episodes of bloodstream infection were defined as positive blood cultures between ICU admission and up to 5 days after discharge to the ward that patients are stepped down to. Cultures from the same patient within 14 days of the original isolate were considered duplicates and excluded. Clinically significant SE-BSI cases were defined as non-duplicate isolates judged by contemporary multidisciplinary interpretation at the time of blood culture results. Cases of positive blood cultures deemed not clinically significant (contaminant) did not meet the definition of study outcome. Central-line associated bloodstream infections (CLA-BSI) were defined as those occurring in a patient who had a central line within 48 h before infection. Episodes related to multiple species were defined as polymicrobial.

Secondary outcomes were CLA-BSI, the proportion of ST2 isolates, *qacA/B* positivity, chlorhexidine tolerance (MIC ≥8 mg/L), and mupirocin resistance. Other genetic determinants were: *mecA, ica* locus, genomic alignment with MRSA-associated plasmid pTW20_1, the copper and mercury resistance element (COMER), and arginine catabolic mobile element (ACME).

Statistical analysis

We summarised medians and IQRs of annual ICU activity, case-mix, and care levels from the two study sites and other Scottish ICUs. Time-averaged differences of clinical characteristics between sites and pre-intervention versus post-intervention were assessed using Mann-Whitney tests or *t*-tests. Differences in study characteristics and antibiotic use over time were evaluated using multilevel generalised additive models (appendix p 4).

To ensure convenience samples of typed and sequenced isolates were representative, we compared resistance phenotypes of untyped, typed, and sequenced isolates using item response theory models (appendix pp 4–5). Differences in isolate phenotypic and genotypic characteristics between sites and pre-intervention versus post-intervention were assessed using Mann-Whitney tests (pTW20_1 alignment scores) and χ^2 tests (proportion of isolates carrying specific genes or mobile genetic elements). $p<0{\cdot}05$ was considered to indicate a statistically significant difference.

To estimate the effect of de-escalation on primary outcomes, we used a before-after-control-impact time-series (BACIT) design (interrupted time-series analyses or, with minor alterations, difference-in-differences).[17] In traditional before-after-control-impact (BACI) designs the effect is inferred post-intervention in relation to a control of a discordant scenario after the intervention. As a variation of this design, in this study the period to infer the effect of de-escalation is before the intervention period, during which the intervention site (ICU one) and control site (ICU two) had discordant decontamination policies, whereas in the after intervention period both ICUs used the same targeted decontamination policy, which was used by ICU two throughout the study. Furthermore, the BACIT design combines time-series analysis with underlying BACI, which accounts for pre-intervention and post-intervention trends over time between the two ICUs, such that any time-varying ecological effects are considered. To assess the sensitivity of findings to model assumptions, we analysed MRSE-BSI incidence density, incorporating median length of stay as a representative covariate of illness severity. We modelled the relationship between MRSE-BSI incidence densities and volumes of chlorhexidine solution and mupirocin ointment prescribed using Poisson

regression with log-link, log OBDs as the offset for a linear effect for time, and a random intercept for month of the year to account for potential seasonality. The probability that an MRSE-BSI case belonged to ST2, carried the *qacA/B* gene, or was mupirocin-resistant was studied in a BACI design without the time-series element, given the relatively short timeframe over which ICU two isolates were typed. All models were fit in a Bayesian framework and are described in the appendix (pp 3–4). Analyses were done in R (version 4.3.1), with models fit in Stan through the R package brms (version 2.21.0).

No formal sample size calculation was done in this natural experiment to avoid risks of post-hoc power calculation.[18] Statistically significant differences were judged based on the ranges of uncertainty (credible intervals [CrIs]) associated with study estimates derived from temporal trends. In comparative studies, estimates with overlapping ranges of uncertainty were deemed to be feasibly indistinguishable.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between July 1, 2009, and Feb 28, 2022, *S epidermidis* was identified in 334 (45%) of 735 bloodstream infections in ICU one (intervention site), of which 197 occurred before the de-escalation intervention in Feb 1, 2019, and *S epidermidis* was identified in 167 (60%) of 278 bloodstream infections in ICU two (control site). 188 (38%) of 501 SE-BSI were CLA-BSI (105 [31%] of 334 in ICU one; 83 [50%] of 167 in ICU two) and 64 (13%) were polymicrobial (49 [15%] of 334 in ICU one; 15 [9%] of 167 in ICU two). 501 (83%) of 603 total bloodstream infection-causing coagulase-negative Staphylococci that were speciated were *S epidermidis*.

Post-surgery admissions and invasive ventilation or level 3 care requirements were significantly lower in ICU one than in ICU two, as was length of stay (table 1). Study ICUs had activity levels, patient demographics, and standardised mortality ratios representative of the national average (table 1; appendix p 6).[13,19]

In both ICU one and ICU two, MRSA carriage at admission screening (0·5 per 1000 OBDs and 0·8 per 1000 OBDs, respectively) and the proportion of *S aureus* bloodstream infections due to MRSA (eight [6%] of 127 infections and three [6%] of 48 infections, respectively) were low throughout the study period.

Monthly chlorhexidine gluconate 4% solution use in ICU one was reduced from 16·0 L (IQR 13·5–19·0) to 2·0 L (IQR 0·8–4·0) after de-escalation, and monthly mupirocin 2% ointment use was reduced from 79·0 g (IQR 57–110) to 0 g (0–0; figure 1A, B). There were no major changes in antibiotic use coinciding with de-escalation in ICU one (appendix p 7). With respect to trends of ICU inpatient activity, after onset of the COVID-19 pandemic (March 1, 2020) there was an activity surge as measured by OBDs in both ICUs (figure 1C), reflected in increased SE-BSI total cases during this period (figure 1E). Monthly and yearly counts of SE-BSI showed no evidence of a seasonal trend (figure 1D, E). We recorded annual bloodstream infections from major bacterial and fungal causes normalised per 1000 OBDs in relation to SE-BSI (figure 1F). An early increase in SE-BSIs in relation to other bloodstream infections was observed in ICU one in 2013, preceding de-escalation by 6 years.

Incidence densities of all bloodstream infections in ICU one after de-escalation and at the end of the study were similar to bloodstream infection incidence at the start of the study and immediately before de-escalation, showing no increase in ICU one coinciding with de-escalation (table 2; figure 2A). The stability of all bloodstream infection trends throughout the study period was observed on a backdrop of reduced standardised mortality and ventilation requirements after de-escalation, but a stable length of stay (table 1). The incidence densities of all bloodstream infections in ICU two was equally stable over the study period (table 2; figure 2A). The proportion of CLA-BSI was higher after de-escalation compared with the pre-intervention period (26·14, 95% CrI 0·00–50·00 *vs* 44·44, 29·12–57·64; p=0·0011) but attributable to a rise in CLA-BSI only during the COVID-19 pandemic. We found no significant difference in the overall proportion of CLA-BSI between ICU one and ICU two (33·33, 95% CrI 11·11–50·00 *vs* 42·86, 0·00–66·67; p=0·17).

The trend of SE-BSI incidence density did not change significantly before or after de-escalation in ICU one and ICU two (table 2; figure 2B), despite the rise in SE-BSI counts during the COVID-19 pandemic (figure 1D).

However, prMRSE-BSI in ICU one immediately before de-escalation decreased significantly 37 months after de-escalation to targeted decolonisation (from 89·2%, 95% CrI 77·8–96·5 to 56·7%, 34·3–77·5%), whereas prMRSE-BSI in ICU two at the equivalent time points was similar (table 2; figure 2C). MRSE-BSI incidence density in ICU one, but not in ICU two, was estimated to have significantly declined by the end of the study compared with immediately before de-escalation (from 10·4 cases per 1000 OBDs, 95% CrI 7·2–15·4 to 4·3 cases per 1000 OBDs, 2·5–6·7; table 2; figure 2D). In sensitivity analysis, inclusion of length of stay did not affect the trends of MRSE-BSI incidence (appendix p 8) compared with the model that did not include this variable (figure 2D). Posterior summaries for all bloodstream infection incidence, SE-BSI incidence, and MRSE-BSI (percentage probability and incidence) are reported in the appendix (pp 16–17).

Furthermore, we found that the incidence density of MRSE-BSI was positively associated with chlorhexidine volumes prescribed in ICU one (figure 2E). At the maximum amount of chlorhexidine use (33 L in December, 2009), MRSE-BSI incidence density was 13·2 cases per 1000 OBDs (95% CrI 6·6 to 23·4) compared with 3·17 cases

	ICU one (intervention site)				ICU two (control site)	p value (ICU one *vs* ICU two)	Scottish intensive and combined critical care units median
	Universal decolonisation	Targeted decolonisation	p value (universal *vs* targeted)	Overall			
Funded level 3 beds, median (range)	10·50 (9·00–12·50)	15·50 (14·25–18·25)	0·0010	11·75 (9·75–14·25)	8·00 (7·5–8·00)	<0·0001	7·34 (6·70–8·22)
Trained nurses (full time equivalent per bed)*	7·00 (6·87–7·00)	6·10 (6·00–6·10)	0·0045	7·00 (6·50–7·00)	5·63 (5·50–5·75)	<0·0001	6·1 (5·7–6·3)
Admissions per year	714·5 (668·0–752·3)	1346·0 (876·5–1461·0)	0·0081	742·0 (674·3–923·8)	386·0 (375·5–419·0)	<0·0001	616·0 (519·4–656·8)
Occupied bed days per year	2986·0 (2831·0–3271·0)	5624·0 (3727·0–6511·0)	0·0083	3202·0 (2844·0–3922·0)	1907·0 (1795·0–2019·0)	<0·0001	2545·0 (2022·0–2623·0)
Bed occupancy (%)	72·30 (69·78–77·33)	70·50 (61·25–73·45)	0·38	72·25 (67·83–76·20)	69·90 (60·93–70·98)	0·034	73·00 (68·54–74·15)
Age at admission, years (mean [SD])	56·90 (0·57)	57·25 (2·06)	0·95	57·00 (1·11)	55·57 (0·93)	0·0014	58·61 (1·60)
Admissions female (%)	40·00 (39·00–41·20)	40·70 (38·60–42·35)	0·81	40·20 (39·00–41·20)	42·00 (39·00–43·00)	0·26	41·95 (40·88–43·18)
Comorbidities (%)†‡	21·00 (14·10–24·30)	17·10 (15·60–19·10)	0·38	19·35 (15·23–23·33)	21·00 (16·50–23·50)	0·70	15·00 (14·00–16·00)
Source of admission (%)§							
Operating room	..	..	..	26·0 (24·0–31·0)	41·0 (40·0–44·0)	0·0006	46·0 (37·0–50·0)
Emergency department	..	..	..	18·0 (17·0–22·0)	22·0 (20·0–23·0)	0·069	20·0 (19·0–22·0)
Ward	..	..	..	21·0 (12·0–25·0)	8·0 (6·0–8·0)	0·0012	15·0 (13·0–15·0)
HDU	..	..	..	13·8 (13·4–17·2)	19·0 (18·0–20·0)	0·0035	9·0 (8·0–12·0)
Specialist ICU in same hospital	..	..	..	1·0 (1·0–2·0)	0·0 (0·0–0·0)	0·0006	0·0 (0·0–0·0)
Other hospital ICU	..	..	..	1·0 (1·0–2·0)	3·0 (2·0–4·0)	0·0087	2·0 (2·0–3·0)
Other hospital non-ICU	..	..	..	11·0 (9·0–12·0)	3·0 (2·0–3·0)	0·0006	4·0 (4·0–6·0)
Highest level of support on admission¶							
Level 3—ICU care	79·20 (72·58–86·33)	53·00 (52·00–77·00)	0·070	77·00 (71·60–83·55)	91·00 (88·51–92·80)	0·0001	67·00 (62·50–71·50)
Level 2—HDU care	18·25 (13·40–22·90)	37·00 (22·00–38·00)	0·051	20·90 (14·95–24·60)	8·00 (6·85–10·80)	0·0001	23·00 (19·00–27·00)
Level 1—enhanced care	1·90 (0·75–2·42)	6·50 (1·00–7·00)	0·22	2·00 (1·00–2·95)	0·20 (0·00–0·90)	0·0013	8·00 (6·50–10·00)
Level 0—acute ward care	1·00 (0·00–2·02)	3·00 (0·00–3·50)	0·27	1·00 (0·00–2·25)	0·00 (0·00–0·00)	0·0016	1·00 (0·90–1·50)
Organ support (proportion of admissions)							
Invasive ventilation	72·50 (66·75–74·0)	45·00 (39·75–64·50)	0·016	70·00 (59·25–74·00)	85·50 (82·75–89·50)	0·0001	61·00 (56·50–64·50)
Vasoactive or anti-arrhythmic (or both)	52·50 (50·75–61·5)	63·0 (51·50–71·50)	0·25	54·00 (50·75–63·25)	62·00 (57·75–64·25)	0·087	49·50 (46·00–52·20)
Renal replacement therapy	15·50 (13·75–16·00)	10·50 (9·25–12·50)	0·0070	14·50 (11·75–16·00)	15·00 (12·00–16·50)	0·78	8·00 (8·00–10·50)
CLA-BSI (%)	26·14 (0·00–50·00)	44·44 (29·12–57·64)	0·0011	33·33 (11·11–50·00)	42·86 (0·00–66·67)	0·17	..
Length of stay (days)	1·90 (1·78–2·13)	2·30 (2·00–2·67)	0·062	2·00 (1·87–2·20)	2·35 (2·20–2·62)	0·0007	2·10 (2·10–2·30)
Recalibrated standardised mortality rate‖**	1·01 (0·91–1·09)	0·83 (0·77–0·87)	0·024	0·91 (0·83–1·04)	0·97 (0·90–1·01)	0·51	0·89 (0·87–0·91)

Data are annual medians (IQR), unless otherwise indicated. All data were reported for 2009–22, unless otherwise stated. All data were obtained from the SICSAG annual reports and the Public Health Scotland acute hospital activity information.[13,19] APACHE II=Acute Physiology and Chronic Health Evaluation II. CLA-BSI=central-line associated bloodstream infection. HDU=high-dependency unit. ICU=intensive care unit. SICSAG=Scottish Intensive Care Society Audit Group. *Data not reported in 2020 due to variable reporting format as per the SICSAG. †The following comorbidities were recorded: severe respiratory disease, image-proven cirrhosis; portal hypertension; immunosuppression; very severe cardiovascular disease; metastatic disease; hepatic encephalopathy; biopsy-proven cirrhosis; chronic renal replacement; lymphoma; chronic leukaemia; acute leukaemia; and AIDS. ‡Data reported for 2012–21. §Data reported for 2009–15. ¶Level of support: level 3, requiring advanced respiratory support alone or monitoring and support for two or more organ systems; level 2, detailed observation or intervention including support for a single failing organ system or post-operative care and those stepping down from higher levels of care; level 1, risk of condition deteriorating, or those recently relocated from higher levels of care where needs can be met on an acute ward with additional advice and support from the critical care team; level 0, needs can be met on an acute ward. ‖Standardised mortality rate is observed mortality/expected mortality, where expected mortality is predicted from the APACHE II severity of disease score at admission to ICU; the recalibrated standardised mortality rate was based on a recalibrated APACHE II score for Scottish ICUs. **Data reported for 2012–22 (standard APACHE II model was recalibrated in 2012 from Scottish ICU and combined units based on data for 2009–11).

Table 1: **Characteristics of study sites in relation to national ICU activity**

per 1000 OBDs (1·8 to 4·9) at the lowest level during targeted decolonisation (1 L in December, 2021; slope 0·04, 95% CrI 0·02 to 0·07; appendix pp 16–17). In the same model, no relationship was observed between mupirocin ointment use and MRSE-BSI incidence density (slope –0·02, 95% CrI –0·25 to 0·16; appendix pp 16–17).

We typed SE-BSI cases from ICU one (250 [75%] of 334 isolates) between July 1, 2009, and Feb 28, 2022, and ICU two (67 [57%] of 117 isolates) between Dec 1, 2016, and Jan 31, 2021 (67 [40%] of 167 isolates for total study period). Whole-genome sequencing was applied to 168 ICU one isolates (67% of 250 typed, 50% of 334 total). Coverage of typing and sequencing was sufficient to avoid selection bias despite convenience sampling (appendix pp 9–10). The phylogenetic distribution of study isolates was representative of major STs identified in a global collection (appendix p 12). ST215, notably absent in our collection, was attributable to prosthetic infections in the global dataset.[16]

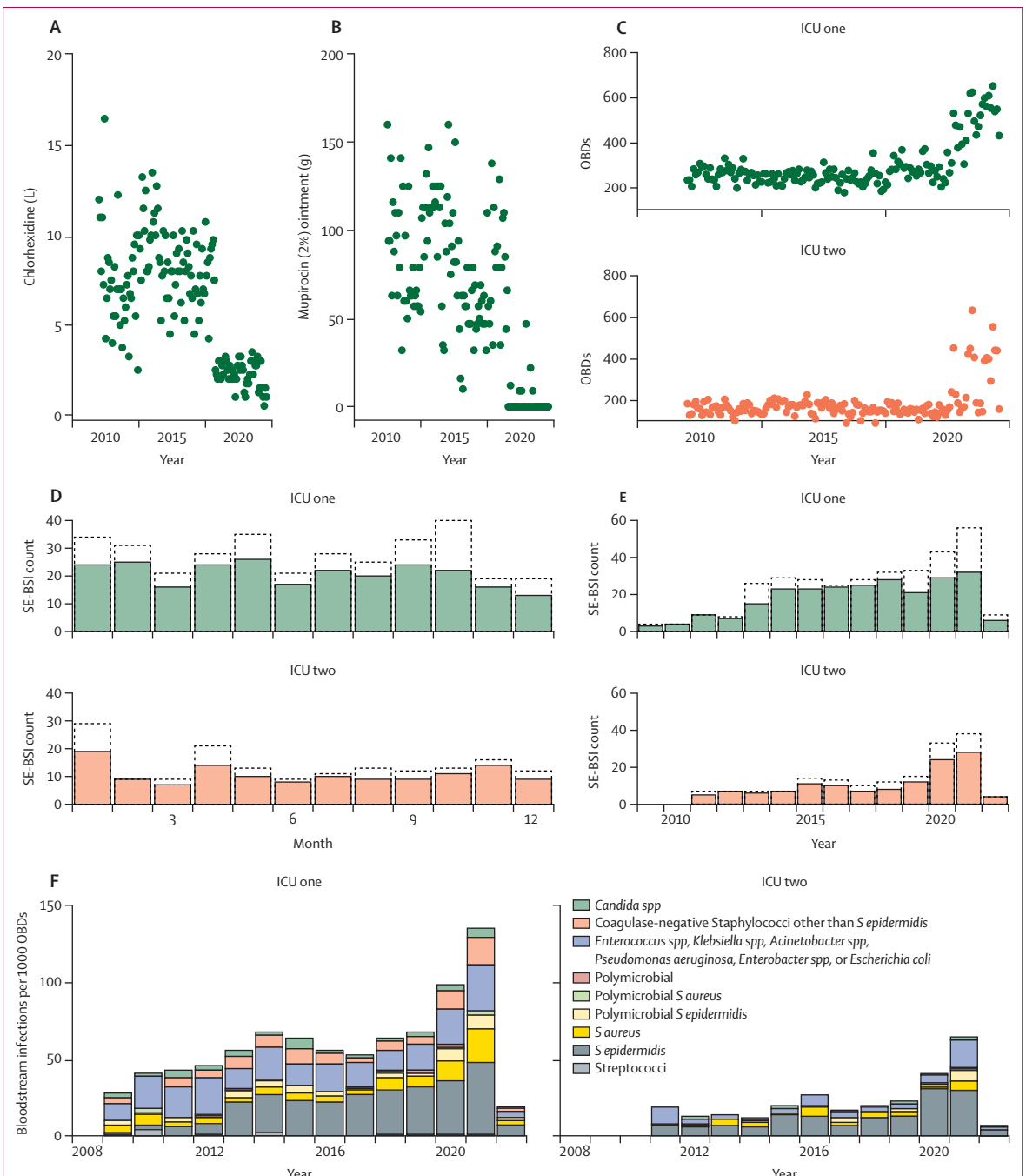

Figure 1: **Chlorhexidine use, intensive care activity, and bloodstream infection aetiological profile over the study period**
Monthly volume of chlorhexidine gluconate 4% solution (A) and monthly amount of mupirocin 2% ointment (B) prescribed in the intervention site (ICU one) over the study period. (C) Monthly activity defined as OBDs in ICU one and control site ICU two. (D, E) Monthly and yearly count of *S epidermidis* cases (dashed bars) and meticillin-resistant *S epidermidis* cases (solid bars). (F) Annual cases bloodstream infections standardised per 1000 OBDs and grouped per cause. Episodes related to multiple non-*S epidermidis* species are identified as polymicrobial and polymicrobial episodes that include *S epidermidis* and *S aureus* are defined as polymicrobial *S epidermidis* and polymicrobial *S aureus*, respectively. OBDs=occupied bed-days. *S aureus*=*Staphylococcus aureus*. *S epidermidis*=*Staphylococcus epidermis*. SE-BSI=*S epidermidis* bloodstream infection.

121 (38%) of 317 of all typed isolates were attributed to the hospital-acquired multidrug resistant lineage ST2 (figure 3; appendix pp 18–21). The SNP-based genetic distance of ST2 isolates from ICU one in relation to reference strain RP62A was 33–61, suggesting high phylogenetic relatedness within this lineage (appendix pp 18–21). Other multidrug-resistant

	Start of study (July 1, 2009)	Immediately before intervention (Jan 31, 2019)	End of study (Feb 28, 2022)
Incidence density of all bloodstream infections			
ICU one	16·2 (13·2–19·8)	20·2 (16·5–24·3)	20·5 (16·3–25·4)
ICU two	14·6 (10·4–19·5)	13·7 (9·8–18·5)	14·6 (10·4–19·5)
Incidence density of SE-BSI			
ICU one	9·02 (3·20–12·51)	14·62 (10·70–19·20)	9·00 (6·42–12·10)
ICU two	6·45 (3·81–10·02)	7·92 (5·35–11·04)	6·27 (4·27–8·78)
Percentage probability of MRSE			
ICU one	71·2% (44·9–90·1)	89·2% (77·8–96·5)	56·7% (34·3–77·5)
ICU two	91·0% (70·1–99·2)	67·6% (41·3–88·4)	79·1% (56·0–93·8)
Incidence density of MRSE			
ICU one	2·3 (1·4–3·5)	10·9 (7·2–15·4)	4·3 (2·5–6·7)
ICU two	1·3 (0·6–2·4)	5·3 (2·9–8·7)	3·7 (1·9–6·4)

Data are incidence density of cases per 1000 occupied bed days (95% credible interval), unless otherwise indicated. ICU=intensive care unit. MRSE=meticillin-resistant *S epidermidis*. *S epidermidis*=*Staphylococcus epidermis*. SE-BSI=*S epidermidis* bloodstream infection.

Table 2: **Summary of primary outcome estimates**

STs included ST5 (19 [8%] of 250 in ICU one; three [4%] of 67 in ICU two) and ST48 (eight [3%] of 250 in ICU one, 0 in ICU two; figure 3; appendix pp 18–21). Representation of ST23, which is associated with linezolid and rifampicin resistance, was minimal (two [1%] of 250 in ICU one).[10,20] In ICU one, the proportion of isolates that belonged to any multidrug-resistant ST declined from 113 (70%) of 162 during universal decolonisation to 22 (25%) of 88 during targeted decolonisation (χ^2 45·98; p<0·0001), whereas meticillin-sensitive sequence types increased (χ^2 11·54; p=0·0007), including ST19 (two [1%] of 162 isolates to eight [9%] of 88 isolates) and ST73 (four [2%] of 162 isolates to six [7%] of 88 isolates; figure 4). In ICU one, de-escalation to targeted decolonisation was associated with substantially higher sequence type diversity. We identified 18 STs during universal decolonisation (116 months) and 39 STs during targeted decolonisation (36 months), of which 25 were undetected during universal decolonisation (appendix pp 18–21). In ICU two, five (23%) of 22 and 13 (29%) of 45 isolates belonged to any multidrug-resistant ST during each study period, respectively (χ^2 0·28; p=0·59; figure 4).

During universal decolonisation in ICU one, the probability of SE-BSI cases belonging to dominant clone ST2 was significantly higher in ICU one (57·8%, 95% CrI 34·8–78·3) than ICU two (13·7%, 95% CrI 3·0–33·6) as determined in BACI studies (figure 5A). De-escalation to targeted decolonisation in ICU one significantly reduced the probability of ST2 SE-BSI from 57·8% to 15·8% (95% CrI 5·2–31·6), whereas in ICU two ST2 prevalence was stable (pre-intervention 13·7%, 95% CrI 3·0–33·6, post-intervention 22·0%, 7·7–42·4; figure 5A).

A larger proportion of typed ICU one isolates had higher chlorhexidine MICs (≥8mg/L) during universal decolonisation than after de-escalation (109 [67%] of 162 isolates *vs* 30 [34%] of 88 isolates; χ^2 25·45; p<0·0001), but not significantly higher than ICU two in the pre-intervention period (12 [55%] of 22 isolates; χ^2 1·40; p=0·24; figures 3, 4). Consistently, in BACI analyses, the percentage of *qacA/B*-positive isolates in ICU one was higher during universal decolonisation (85·4%, 95% CrI 74·4–93·7) than during targeted decolonisation (47·3%, 28·3–61·3; figure 5B). However, CrIs of the percentage of *qacA/B*-positive isolates in ICU one overlapped with that in ICU two (pre-intervention 55·1%, 95% CrI 32·6–77·4, post-intervention 70·8%, 52·0–86·7); figure 5B). Mupirocin resistance in ICU one was not significantly reduced after de-escalation (pre-intervention 23·9%, 95% CrI 10·7–40·0, post-intervention 13·6%, 4·8–26·6) and was similar to ICU two (3·2%, <0·1–12·3 *vs* 1·8%, <0·1–7·1; figure 5C). Posterior summaries for the proportion of ST2 SE-BSI, *qacA/B* carriers, and mupirocin resistance are reported in the appendix (p 22).

The prevalence of *mecA*-positive isolates was higher in ICU one during universal decolonisation compared with ICU two (136 [84%] of 162 isolates *vs* 13 [59%] of 22 isolates; χ^2 6·84; p=0·0092) and when compared with the de-escalation period in ICU one (136 [84%] of 162 isolates *vs* 45 [51%] of 88 isolates; χ^2 21·82; p<0·0001; figure 4). Reduced susceptibility to chlorhexidine was often observed in isolates that co-carried *qacA/B* with antibiotic resistance genes on SCC*mec* (figure 3). Therefore, isolates with higher chlorhexidine MIC were more represented among globally distributed MRSE STs (figure 3). These isolates carried biofilm formation genes (*ica* locus) and mobile genetic elements associated with multidrug resistance and increased fitness in *S aureus* and *S epidermidis* (COMER, pTW20_1) more frequently than isolates with lower chlorhexidine MIC (figure 3).[3,21,22] Within genome-sequenced isolates, there was a higher proportion of COMER during universal versus targeted decolonisation (12 [15%] of 80 isolates *vs* three [3%] of 88 isolates; χ^2 6·63; p=0·010; figure 4) with all COMER-positive isolates during universal decolonisation belonging to ST2 and during targeted decolonisation to ST54.[12] Isolates from universal decolonisation had significantly larger regions mapped to MRSA plasmid pTW20_1 compared with isolates from targeted decolonisation (maximum alignment score 4588, IQR 4257–8163 *vs* 3155, 1648–4942; Mann-Whitney p<0·0001; figure 4; appendix pp 18–21). The *ica* locus was found in 82 (71%) of 115 MRSE isolates (including ST2, ST48, and ST23) compared with 12 (23%) of 53 non-MRSE sequenced isolates (χ^2 34·86; p<0·0001), and in 55 (69%) of 80 isolates during universal decolonisation versus 39 (44%) of 88 isolates during targeted decolonisation (χ^2 9·72; p=0·0018; figure 4). Conversely, ACME was less represented during universal decolonisation than in targeted decolonisation (21 [26%] of 80 isolates *vs* 36 [41%] of 88 isolates; χ^2 5·77; p=0·016) and was distributed among a range of STs (figure 3).

Discussion

In this retrospective, controlled time-series analysis, we found that de-escalation from universal to targeted skin and nasal decolonisation in an ICU population with low MRSA

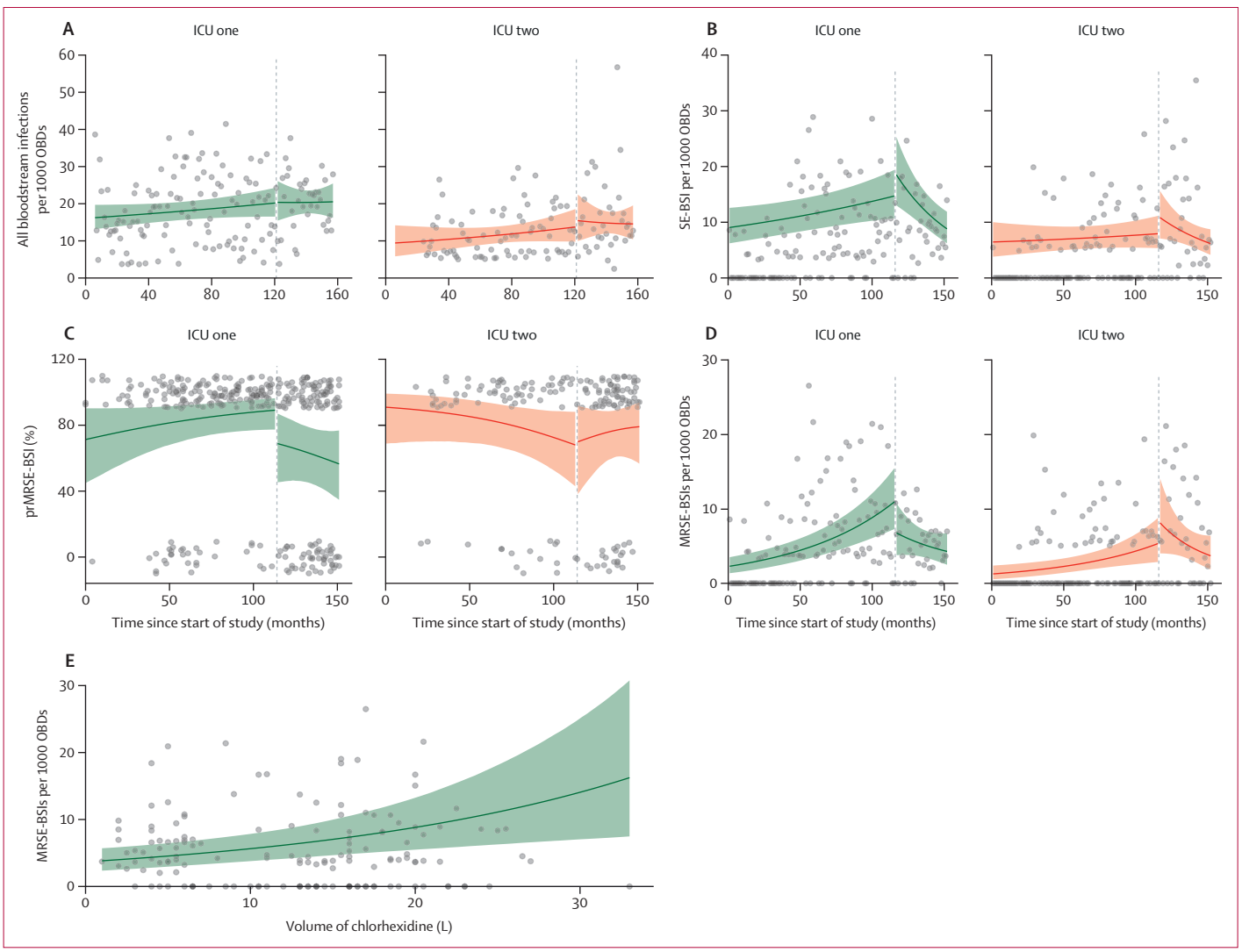

Figure 2: **Effects of universal *vs* targeted decolonisation on all bloodstream infections, SE-BSI, and MRSE-BSI**

(A) Incidence density of all bloodstream infections (per 1000 OBDs) over time in ICU one and ICU two. (B) Incidence density of SE-BSIs (per 1000 OBDs) over time. (C) prMRSE-BSI over time. (D) Incidence density of MRSE-BSIs measured as MRSE-BSI cases per 1000 OBDs over time. Points have been jittered (random noise has been addedd to the position of each point to visualise true data distribution) to enhance legibility. The vertical line in A–D shows the timepoint at which de-escalation occurred in ICU one. (E) Predicted number of MRSE-BSI cases per 1000 OBDs for each L of chlorhexidine delivered to ICU one in a given month. Solid lines in all panels show the mean posterior predictions and shaded areas show 95% credible intervals. Data points represent the raw input data provided to the model. MRSE=meticillin-resistant *S epidermis*. MRSE-BSI=meticillin-resistant *S epidermidis* bloodstream infection. OBDs=occupied bed-days. prMRSE-BSI=percentage probability that SE-BSI were meticillin resistant. *S epidermidis*=*Staphylococcus epidermis*. SE-BSI=*S epidermidis* bloodstream infection.

incidence was associated with reduced incidence of MRSE-BSI and did not lead to increased incidence density of all bacterial or fungal bloodstream infections. SE-BSI trends were stable throughout the study period despite overall lower standardised mortality rates and ventilation requirements after de-escalation. MRSE-BSI incidence was positively associated with the amount of chlorhexidine used, but not the amount of mupirocin, in the de-escalation intervention site in which universal decolonisation was pursued until early 2019. Trends of chlorhexidine and mupirocin use pre-intervention and post-intervention showed partial collinearity. However, the greater variance of mupirocin use, possibly reflective of measurement imprecision, probably accounts for the absence of association between MRSE-BSI incidence and mupirocin use. In longitudinal genotypic analyses, de-escalation from universal to targeted skin and nasal decolonisation reduced selection of hospital-associated multidrug-resistant STs and increased prevalence of meticillin-sensitive clones such as ST19.[23] De-escalation to targeted decolonisation reduced the prevalence of isolates with higher chlorhexidine MIC, reduced carriage of mobile genetic elements associated with multidrug resistance, and increased fitness. The higher genomic alignment with MRSA plasmid pTW20_1 in isolates with reduced susceptibility to chlorhexidine suggests that intensive chlorhexidine use might promote horizontal

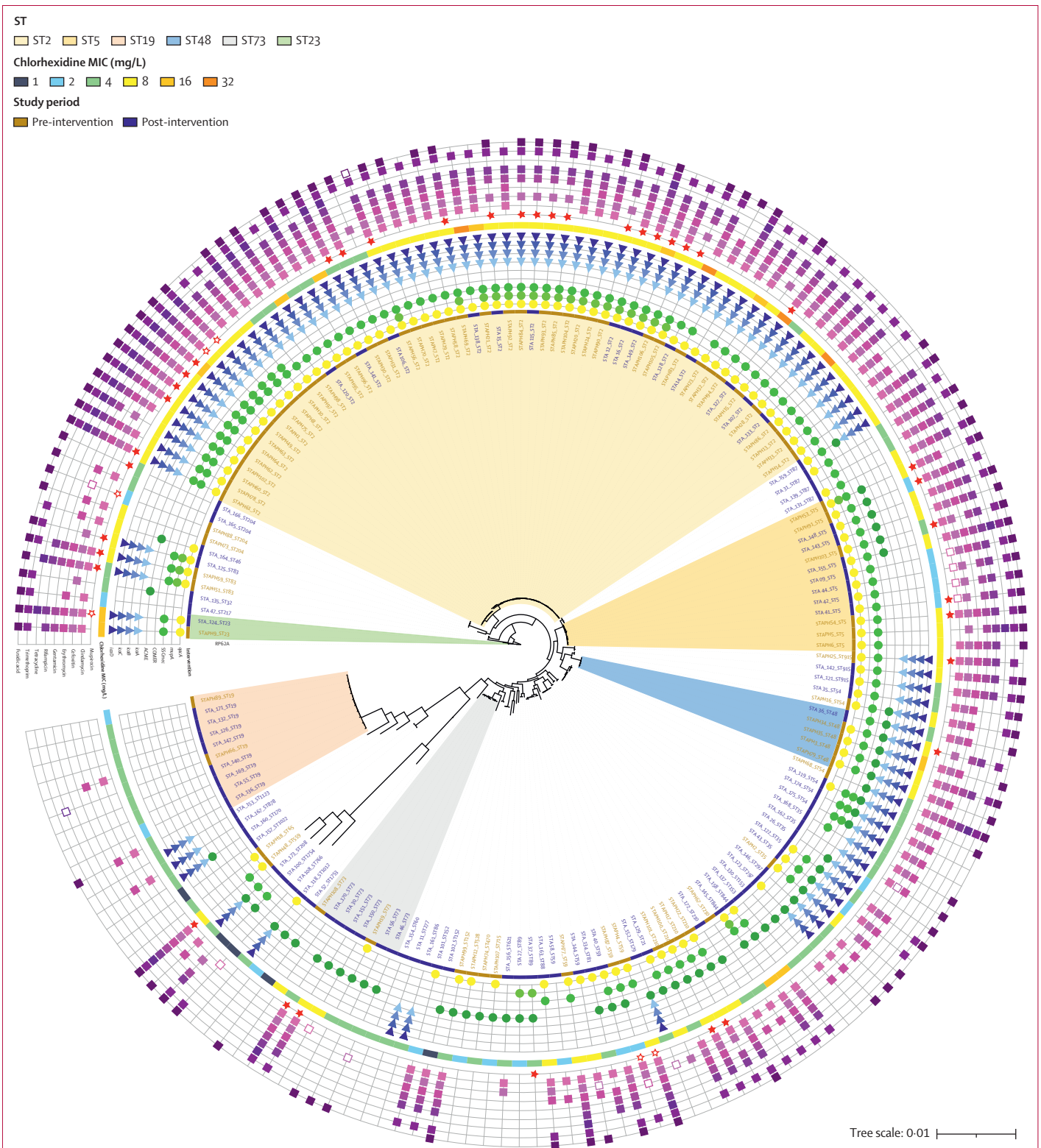

Figure 3: **Clonal structure of *Staphylococcus epidermidis* bacteraemia isolates during universal and targeted decolonisation**
Core-genome phylogenetic tree based on RAxML outputs of 168 isolates from intensive care unit one during the study period. Major STs in relation to *mecA*, *qacA/B*, COMER, ACME, *mupA*, and *ica* locus positivity and chlorhexidine and antibiotic susceptibilities are annotated. Reference strain RP62A is included. ACME=arginine catabolic mobile element. COMER=copper and mercury resistance element. MIC=minimum inhibitory concentration. ST=sequence type.

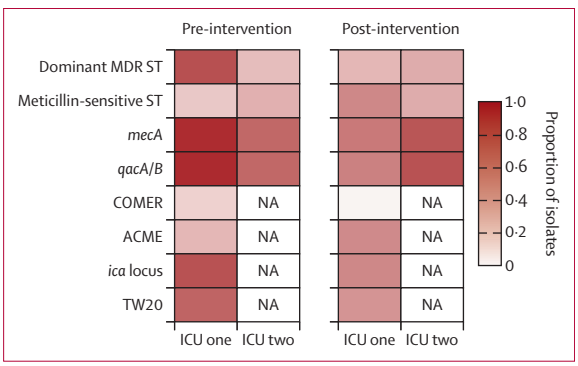

Figure 4: **Clonal distribution and carriage of determinants of multidrug resistance during universal and targeted decolonisation**
Heatmap summarising the total proportion of the most frequent MDR STs, meticillin-sensitive STs, and carriage of genetic determinants of multidrug resistance, *qacA/B* carriage, chlorhexidine tolerance (minimum inhibitory concentration ≥8 mg/mL), and *ica* genes for biofilm production during universal decolonisation (pre-intervention) and targeted decolonisation (post-intervention). The colour intensity gradient in the heatmap key reflects the proportion of values out of the number of isolates analysed for a particular characteristic during the respective study period for ICU one and ICU two. ACME=arginine catabolic mobile element. COMER=copper and mercury resistance element. ICU=intensive care unit. MDR=multidrug resistant. NA=not applicable. ST=sequence type. TW20=maximum alignment scores relative to pTW20_1 sequence FN433597.

gene transfer between *S epidermidis* and *S aureus*. The higher proportion of isolates harbouring the COMER mobile element during universal decolonisation, exclusively represented in ST2 during this period, suggests selection of *S epidermidis* lineages with increased fitness.[22] The finding of lower MRSE-BSI incidence and reduced multidrug-resistant STs under targeted colonisation compared with universal decolonisation in the intervention site was corroborated by comparative measurements with a control ICU with similar activity, patient profiles, antibiotic prescribing practices, and patient care standards but using targeted decolonisation throughout. Here, MRSE-BSI cases during universal decolonisation rose substantially in the intervention site compared with the marginal increase in the control site, despite higher predisposing factors in the control site, namely a higher proportion of CLA-BSIs, requirement for invasive ventilation and level 3 care, and a longer length of stay. Likewise, the probability of SE-BSI cases belonging to dominant multidrug-resistant ST2 during this period was higher than at the control site during the same period. Chlorhexidine MICs and *qacA/B* carriage during the pre-intervention period were similar across the intervention and control sites, notwithstanding the comparatively small sample of typed isolates in the control site and that *qacA/B* expression was not tested in this study.

The longitudinal study design allowed us to compare clinical and genotypic MRSE-BSI profiles from ICUs during long-term implementation of decolonisation strategies and to identify timeframes for reversal of multidrug resistance on de-escalation. Although we corrected for expected temporal behaviour, the observational and quasi-experimental design of our study limits conclusions about causal effects

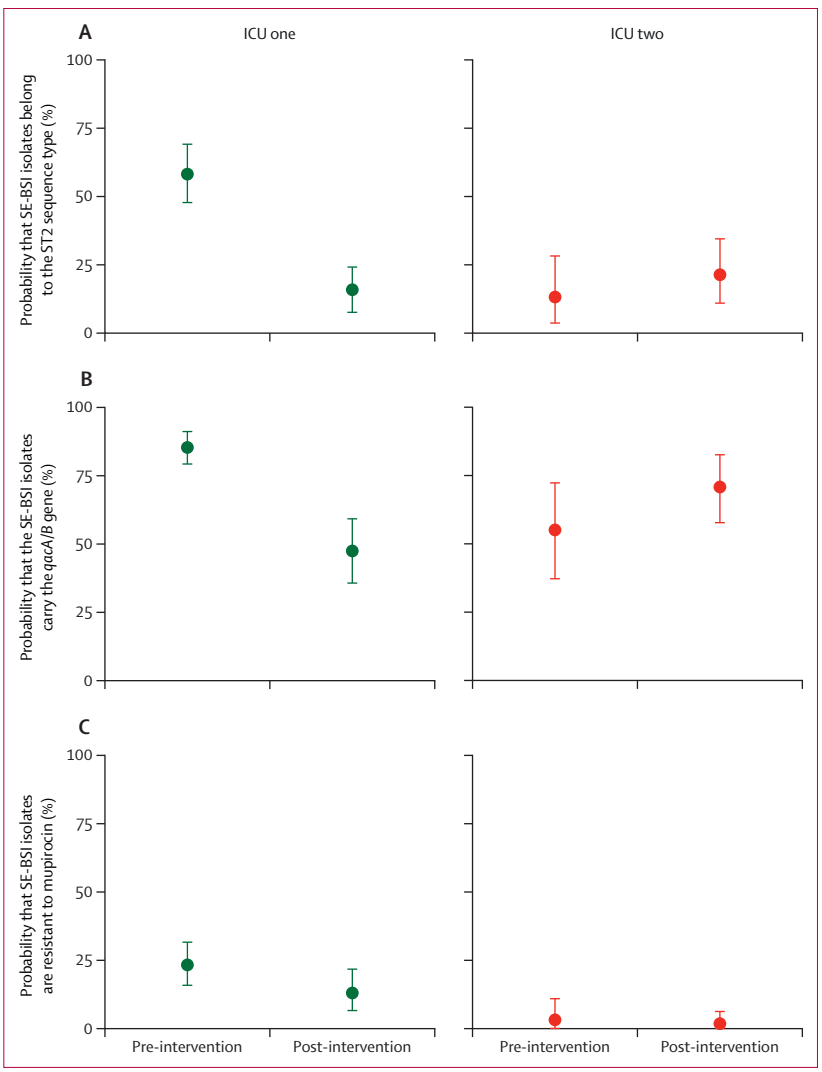

Figure 5: **Probability of *S epidermidis* isolates belonging to multidrug resistance clone ST2, being *qacA/B* carriers, and being mupirocin resistant**
Before-after-control-impact predictions for the probability that SE-BSI belong to the ST2 sequence type (A), carry the *qacA/B* gene (B), and are resistant to mupirocin (C), before (pre-intervention) and after de-escalation of decolonisation (post-intervention) in the intervention site (ICU one) and the control site (ICU two) at the equivalent study periods. Points represent the mean of the posteriors and error bars are 95% credible intervals. ICU=intensive care unit. *S epidermidis*=*Staphylococcus epidermis*. SE-BSI=*S epidermidis* bloodstream infection. ST=sequence type.

of de-escalation as we cannot exclude the confounding effect of unmeasured patient-level and ecological variables, such as line days and use of temporary nursing staff. To mitigate the potential confounding effects of ecological variables, not least the changes in hospital activity and infection control precautions during the COVID-19 pandemic, we used a controlled time-series design, simultaneously adjusting for expected temporal behaviour based on historic pre-intervention data and contemporary control data derived from an ICU site with similar activity and patient profiles, but using targeted decolonisation throughout. Given the inherent advantages of this study design, full multivariable analysis was not done to avoid the risk of statistical overfitting. Nonetheless, a range of surrogate markers of

severe disease were higher in the control site than in the intervention site and thus unlikely to explain the effects on study outcome. Sensitivity analysis integrating length of stay as representative covariate of illness severity showed that this variable did not contribute to MRSE incidence trends. Time-series analysis of antibiotic use in ICU one did not show an association with primary outcomes. Although we cannot exclude the potential confounding effect of patient-level variables, this risk was mitigated by the fact that, during periods of targeted decolonisation, biocide use was limited to decolonisation of MRSA-positive admissions, such that the primary exposure at case level was concordant with the active decolonisation policy.

Universal decolonisation for patients in the ICU has been shown to effectively control staphylococcal CLA-BSI and showed superiority over targeted decolonisation in a randomised controlled trial of 43 hospitals in the USA.[24–26] Superiority of universal decolonisation was also observed in relation to reduction of blood culture contamination.[27] Concerns over reduced susceptibility to chlorhexidine and selection of multidrug resistance and conflicting findings on the effectiveness of chlorhexidine bathing have underpinned controversy around universal decolonisation.[7,28,29] Several studies have focused on the effect of chlorhexidine exposure on chlorhexidine susceptibility of MRSA, reporting conflicting conclusions. Observational studies have shown reduced chlorhexidine susceptibility of *S aureus* with high use of chlorhexidine, albeit with no clonal expansion.[30,31] Randomised controlled trials have shown that susceptibility to chlorhexidine did not decrease with chlorhexidine bathing, notwithstanding short implementation periods.[32] However, the effect of chlorhexidine exposure on multidrug resistance in *S epidermidis* has been neglected. In this study, for the first time to our knowledge, we show reduced selection of MRSE on de-escalation from universal to targeted decolonisation.

We call on ICUs practicing universal skin and nasal decolonisation to appraise risks and benefits of this practice in the context of their effect on the emergence and spread of MRSE. Some hospitals have adopted octenidine as a replacement for chlorhexidine following concerns of chlorhexidine-tolerant MRSA clones.[33] Nonetheless, efforts to rationalise skin decolonisation practices should extend beyond octenidine, particularly in view of the similarity in chemical structures between chlorhexidine and octenidine. The findings of our study should have implications in surgical settings, particularly in view of WHO guidelines on surgical site infection prevention supporting targeted decolonisation of *S aureus* carriers undergoing cardiothoracic and orthopaedic surgery.[34] A clinical trial on all-cause bacteraemias in medical and surgical units showed that universal chlorhexidine bathing did not significantly reduce multidrug-resistant organisms in non-critical-care patients.[35] Findings should also affect implementation of universal MRSA admission screening with subsequent decolonisation for MRSA-positive patients admitted to all acute specialties. There remains debate worldwide over the value of universal MRSA screening versus a risk-based targeted approach, such as that adopted in Scotland.

In conclusion, regulation of biocide use might prevent unintended harms. Future studies should investigate the effects of universal decolonisation on other multidrug-resistant organisms. In this context, alternative strategies, such as targeted decolonisation and microbiota repopulation strategies should be considered to minimise selection of multidrug resistant lineages of skin and mucous membrane colonisers.

Contributors

KH, CAM, MO, and TL conceived the hypothesis, aims, and design of the study. AR, SA, IN, IO, MO, and DG contributed to the genomic studies and phylogenetic analyses. SS did the multilocus sequence typing analysis. SA and TL extracted and analysed the publicly available intensive care unit (ICU) auditing data. DR did the before-after time series analysis and TL provided additional analyses. DA and TL contributed analysis of data structure informing assumptions underpinning the time series analysis. BW and TR extracted and coordinated isolate collection, bloodstream infection, and antimicrobial susceptibility data for the intervention site. BJP, VA, and SJR extracted and coordinated isolate collection, bloodstream infection, and antimicrobial susceptibility data for the control site. IM contributed ICU activity data. KH, SS, SA, IO, BJP, TL, and BW directly accessed and verified the underlying data reported. KH acquired funding, supervised the study, and oversaw interpretation of results. KH, DR, and TL wrote the original manuscript draft. All authors reviewed and edited the manuscript. All authors had access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The genome data of the newly sequenced isolates for this study will be available via National Center for Biotechnology Information GenBank with publication of this Article (Bioproject unique identifier PRJNA982045). Previously sequenced genomes are already available via GenBank [PRJNA574294]). Isolate metadata have been included in the appendix (pp 18–21). R codes will be available on publication at https://github.com/DeonRoos/002—AntiRes. Requests for anonymised datasets can be made by contacting the corresponding author.

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References

1 Burke TL, Rupp ME, Fey PD. *Staphylococcus epidermidis*. *Trends Microbiol* 2023; **31:** 763–64.

2 Both A, Huang J, Qi M, et al. Distinct clonal lineages and within-host diversification shape invasive *Staphylococcus epidermidis* populations. *PLoS Pathog* 2021; **17:** e1009304.

3 Jamet A, Guglielmini J, Brancotte B, et al. High-resolution typing of *Staphylococcus epidermidis* based on core genome multilocus sequence typing to investigate the hospital spread of multidrug-resistant clones. *J Clin Microbiol* 2021; **59:** e02454-20.

4 Tolo I, Thomas JC, Fischer RSB, Brown EL, Gray BM, Robinson DA. Do *Staphylococcus epidermidis* genetic clusters predict isolation sources? *J Clin Microbiol* 2016; **54:** 1711–19.

5 Zamudio R, Oggioni MR, Gould IM, Hijazi K. Time for biocide stewardship? *Nat Microbiol* 2019; **4:** 732–33.

6 Méric G, Miragaia M, de Been M, et al. Ecological overlap and horizontal gene transfer in *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Genome Biol Evol* 2015; **7:** 1313–28.

7 Abbood HM, Hijazi K, Gould IM. Chlorhexidine resistance or cross-resistance, that is the question. *Antibiotics (Basel)* 2023; **12:** 798.

8 Batra R, Cooper BS, Whiteley C, Patel AK, Wyncoll D, Edgeworth JD. Efficacy and limitation of a chlorhexidine-based decolonization strategy in preventing transmission of methicillin-resistant *Staphylococcus aureus* in an intensive care unit. *Clin Infect Dis* 2010; **50:** 210–17.

9 Furi L, Ciusa ML, Knight D, et al. Evaluation of reduced susceptibility to quaternary ammonium compounds and bisbiguanides in clinical isolates and laboratory-generated mutants of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2013; **57:** 3488–97.

10 Lee JYH, Monk IR, Gonçalves da Silva A, et al. Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*. *Nat Microbiol* 2018; **3:** 1175–85.

11 Hijazi K, Mukhopadhya I, Abbott F, et al. Susceptibility to chlorhexidine amongst multidrug-resistant clinical isolates of *Staphylococcus epidermidis* from bloodstream infections. *Int J Antimicrob Agents* 2016; **48:** 86–90.

12 Holden MT, Lindsay JA, Corton C, et al. Genome sequence of a recently emerged, highly transmissible, multi-antibiotic- and antiseptic-resistant variant of methicillin-resistant *Staphylococcus aureus*, sequence type 239 (TW). *J Bacteriol* 2010; **192:** 888–92.

13 Public Health Scotland. Acute hospital activity and NHS beds information (annual). https://publichealthscotland.scot/publications/show-all-releases?id=20521 (accessed Jan 21, 2025).

14 National Services Scotland. Protocol for clinical risk assessment (CRA) meticillin resistant *Staphylococcus aureus* (MRSA) screening national rollout in Scotland. Dec 1, 2019. https://www.nss.nhs.scot/publications/protocol-for-clinical-risk-assessment-cra-meticillin-resistant-staphylococcus-aureus-mrsa-screening-national-rollout-in-scotland/ (accessed Jan 21, 2025).

15 Thomas JC, Vargas MR, Miragaia M, Peacock SJ, Archer GL, Enright MC. Improved multilocus sequence typing scheme for *Staphylococcus epidermidis*. *J Clin Microbiol* 2007; **45:** 616–19.

16 Månsson E, Bech Johannesen T, Nilsdotter-Augustinsson Å, Söderquist B, Stegger M. Comparative genomics of *Staphylococcus epidermidis* from prosthetic-joint infections and nares highlights genetic traits associated with antimicrobial resistance, not virulence. *Microb Genom* 2021; **7:** 000504.

17 Wauchope HS, Amano T, Geldmann J, et al. Evaluating impact using time-series data. *Trends Ecol Evol* 2021; **36:** 196–205.

18 Hoenig JM, Heisey DM. The Abuse of Power. *Am Stat* 2001; **55:** 19–24.

19 Scottish Intensive Care Society Audit Group. Audit of critical care in NHS Scotland. Annual Reports. https://www.sicsag.scot.nhs.uk/publications/main.htm (accessed Jan 21, 2025).

20 Mendes RE, Deshpande LM, Farrell DJ, Spanu T, Fadda G, Jones RN. Assessment of linezolid resistance mechanisms among *Staphylococcus epidermidis* causing bacteraemia in Rome, Italy. *J Antimicrob Chemother* 2010; **65:** 2329–35.

21 Purves J, Thomas J, Riboldi GP, et al. A horizontally gene transferred copper resistance locus confers hyper-resistance to antibacterial copper toxicity and enables survival of community acquired methicillin resistant *Staphylococcus aureus* USA300 in macrophages. *Environ Microbiol* 2018; **20:** 1576–89.

22 Almebairik N, Zamudio R, Ironside C, et al. Genomic stability of composite SCC*mec* ACME and COMER-like genetic elements in *Staphylococcus epidermidis* correlates with rate of excision. *Front Microbiol* 2020; **11:** 166.

23 Ahle CM, Stødkilde K, Poehlein A, et al. Interference and co-existence of staphylococci and *Cutibacterium acnes* within the healthy human skin microbiome. *Commun Biol* 2022; **5:** 923–26.

24 Huang SS, Septimus E, Kleinman K, et al. Targeted versus universal decolonization to prevent ICU infection. *N Engl J Med* 2013; **368:** 2255–65.

25 Gould IM, MacKenzie FM, MacLennan G, Pacitti D, Watson EJ, Noble DW. Topical antimicrobials in combination with admission screening and barrier precautions to control endemic methicillin-resistant *Staphylococcus aureus* in an intensive care unit. *Int J Antimicrob Agents* 2007; **29:** 536–43.

26 Reynolds SS, Woltz P, Keating E, et al. Results of the chlorhexidine gluconate bathing implementation intervention to improve evidence-based nursing practices for prevention of central line associated bloodstream infections dtudy (CHanGing BathS): a stepped wedge cluster randomized trial. *Implement Sci* 2021; **16:** 45.

27 Septimus EJ, Hayden MK, Kleinman K, et al. Does chlorhexidine bathing in adult intensive care units reduce blood culture contamination? A pragmatic cluster-randomized trial. *Infect Control Hosp Epidemiol* 2014; **35** (suppl 3)**:** 17–22.

28 Pittet D, Angus DC. Daily chlorhexidine bathing for critically ill patients: a note of caution. *JAMA* 2015; **313:** 365–66.

29 Kampf G. Acquired resistance to chlorhexidine—is it time to establish an 'antiseptic stewardship' initiative? *J Hosp Infect* 2016; **94:** 213–27.

30 Htun HL, Hon PY, Holden MTG, Ang B, Chow A. Chlorhexidine and octenidine use, carriage of qac genes, and reduced antiseptic susceptibility in methicillin-resistant *Staphylococcus aureus* isolates from a healthcare network. *Clin Microbiol Infect* 2019; **25:** 1154.e1–7.

31 Hardy K, Sunnucks K, Gil H, et al. Increased usage of antiseptics is associated with reduced susceptibility in clinical isolates of *Staphylococcus aureus*. *MBio* 2018; **9:** e00894-18.

32 Denkel LA, Kramer TS, Schwab F, et al. Chlorhexidine and octenidine susceptibility of bacterial isolates from clinical samples in a three-armed cluster randomised decolonisation trial. *PLoS One* 2022; **17:** e0278569.

33 Spencer C, Orr D, Hallam S, Tillmanns E. Daily bathing with octenidine on an intensive care unit is associated with a lower carriage rate of meticillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 2013; **83:** 156–59.

34 Allegranzi B, Bischoff P, de Jonge S, et al. New WHO recommendations on preoperative measures for surgical site infection prevention: an evidence-based global perspective. *Lancet Infect Dis* 2016; **16:** e276–87.

35 Huang SS, Septimus E, Kleinman K, et al. Chlorhexidine versus routine bathing to prevent multidrug-resistant organisms and all-cause bloodstream infections in general medical and surgical units (ABATE Infection trial): a cluster-randomised trial. *Lancet* 2019; **393:** 1205–15.