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Whole-genome sequencing reveals widespread presence of *Staphylococcus capitis* NRCS-A clone in neonatal units across the United Kingdom

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SUMMARY

Objective: Increased incidence of neonatal *Staphylococcus capitis* bacteraemia in summer 2020, London, raised suspicion of widespread multidrug-resistant clone NRCS-A. We set out to investigate the molecular epidemiology of this clone in neonatal units (NNUs) across the UK.
Methods: We conducted whole-genome sequencing (WGS) on presumptive *S. capitis* NRCS-A isolates collected from infants admitted to nationwide NNUs and from environmental sampling in two distinct NNUs in 2021. Previously published *S. capitis* genomes were added for comparison. Genetic clusters of NRCS-A isolates were defined based on core-genome single-nucleotide polymorphisms.
Results: We analysed WGS data of 838 *S. capitis* isolates and identified 750 NRCS-A isolates. We discovered a possible UK-specific NRCS-A lineage consisting of 611 isolates collected between 2005 and 2021. We determined 28 genetic clusters of NRCS-A isolates, which covered all geographical regions in the UK, and isolates of 19 genetic clusters were found in ≥2 regions, suggesting inter-regional spread. Within the NRCS-A clone, strong genetic relatedness was identified between contemporary clinical and incubator-associated fomite isolates and between clinical isolates associated with inter-hospital infant transfer.
Conclusions: This WGS-based study confirms the dispersion of *S. capitis* NRCS-A clone amongst NNUs across the UK and urges research on improving clinical management of neonatal *S. capitis* infection.
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Introduction

Staphylococcus capitis is a member of coagulase-negative staphylococci (CoNS) and part of microbiota on human and animal skins, mucous membranes, and in guts.[1–4] As an opportunistic pathogen, *S. capitis* is a common aetiological agent of late-onset (> 72 h

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of life) sepsis in very-low-birthweight (< 1.5 kg) preterm infants in neonatal intensive care units (NICUs) worldwide.[5] It also causes hospital- and community-acquired infections, such as meningitis, infective endocarditis, osteomyelitis, pneumonia, urinary tract infection, catheter-related bacteraemia, prosthetic joint infection, skin and wound infections, *etc*, in adults (especially those aged ≥65 and/or with suppressed immunity or underlying cardiac disease).[6–11] In England, *S. capitis* was the second most common species among speciated CoNS recovered from normally sterile anatomical sites of infants (under 12 months) between 2010 and 2021, with an increasing annual count of reported cases.[11] A multicentre study of *S. capitis* bloodstream infections in French NICUs relates 50% cases to venous catheters followed by digestive tract, skin, and pulmonary tract.[12]

S. capitis belongs to the Epidermidis cluster group of CoNS (together with *S. epidermidis*, *S. caprae*, and *S. saccharolyticus*)[13] and consists of two subspecies, *capitis* and *urealyticus*, distinguished by DNA homology, urease production, maltose fermentation, fatty acid profiles, and colony morphology.[14] Studies suggest that subspecies *urealyticus* generally has a greater pathogenic potential than subspecies *capitis* for enrichment of genes encoding virulence factors and antimicrobial resistance.[6,10,15] In the early 2010s, the NRCS-A clone of *S. capitis* subsp. *urealyticus* was first discovered in France as a pulsogroup using pulsed-field gel electrophoresis and thought to have emerged in 1952–1986.[5,16] The NRCS-A clone has evolved into three lineages ('proto-outbreak 1', 'proto-outbreak 2', and 'international outbreak'),[16] and 59 genes specific to the NRCS-A clone were identified as markers through genomic comparisons to allow sequence-based detection of this clone.[17]

The NRCS-A clone is resistant/heteroresistant to vancomycin, mostly resistant to fosfomycin, aminoglycosides, and beta-lactams that are commonly used in NICUs, and frequently resistant to fusidic acid.[16,18,19] The NRCS-A clone usually dominates invasive *S. capitis* isolates from NICUs worldwide and has a propensity to persist in NICUs once introduced.[16,19,20] Biofilm formation by *S. capitis* on surfaces of medical device is a risk factor for infections in NICUs,[21] and the NRCS-A clone presents an enhanced ability to form biofilms and withstand desiccation,[22] which would explain the persistence of this clone in NICUs despite strict hygiene measures.[23]

In June–August 2020, clinicians from several London NICUs alerted the UK Health Security Agency (UKHSA, formerly Public Health England) to a suspected increase in *S. capitis* bacteraemia incidence since 2019 without any technical changes in their microbiology laboratories. Comparison of whole-genome sequencing (WGS) data of *S. capitis* isolates obtained from neonatal bacteraemia episodes in London over this period with 118 available *S. capitis* genomes at the UKHSA by September 2020 raised suspicion of wider presence of the NRCS-A clone. Therefore, the UKHSA convened a national incident management team (IMT) and requested microbiology laboratories to voluntarily refer *S. capitis* bacteraemia isolates from neonates to the national reference laboratory for WGS over 12 months starting from February 2021.[24,25] Before this incident response, WGS and molecular typing of nationwide *S. capitis* isolates had not been conducted in the UK. Here, we report the wide distribution of the *S. capitis* NRCS-A clone in neonatal units (NNUs) across the UK and highlight the essential role of WGS in informing clinical management of neonatal *S. capitis* infection.

Materials and methods

Isolate collection

A presumptive NRCS-A isolate was defined as an *S. capitis* isolate that was resistant to any beta-lactam, gentamicin, and fusidic acid, and was susceptible to rifampicin and ciprofloxacin.[24] In response to UKHSA's request, presumptive NRCS-A isolates cultured from normally sterile sites of infants (< 1 year of life) admitted to NNUs across the UK were sent to the UKHSA Reference Laboratory for WGS (n = 355, received from February 2021 through November 2021). Moreover, clinical *S. capitis* isolates referred to the UKHSA from all age groups in the UK (n = 382) and Ireland (n = 3) between August 2005 and October 2021 for routine reference services also underwent WGS to add to our reconstruction of UK *S. capitis* population structure and to determine whether isolates differed between infants and other age groups. Patient data were extracted from referral forms accompanying these 740 clinical isolates.

To investigate possible environmental reservoirs of the NRCS-A clone within NICUs, 20 environmental isolates recovered in two distinct NICUs (> 160 km apart) in the South West of England between August and September 2021 were referred to the UKHSA for WGS. Of these isolates, 18 were sampled from fomites related to neonatal incubators and two were sampled from air (see Supplementary Table 1 for details of sampling sites).

Furthermore, previously published WGS reads of 76 NRCS-A isolates (collected between 1997 and 2015 and comprised of 39 UK and 37 non-UK isolates) were downloaded from the European Nucleotide Archive (ENA)[26] to represent the global population structure of this clone. These isolates were selected to cover all major clades within the international 'outbreak' and two 'proto-outbreak' NRCS-A lineages described by Wirth et al.[16] Genome assemblies of *S. capitis* type strains (subsp. *capitis*: DSM 20326; subsp. *urealyticus* DSM 6717) were downloaded from the NCBI Assembly database (accessions: DSM 20326, GCA_025272975.1; DSM 6717, GCF_002901925.1) for subspecies identification. Supplementary Table 1 provides isolate information of all WGS data used in this study.

Whole-genome sequencing

WGS was conducted on HiSeq 2500 systems (Illumina, San Diego, USA) at the UKHSA Central Sequencing Laboratory for a minimum depth of 40 folds with an in-house paired-end 101-bp sequencing protocol. Low-quality bases were removed from sequencing reads with Trimmomatic, and processed reads were summarised with SeqKit.[27,28] Species identification and contamination were assessed using Kraken2 and Bracken.[29,30] See Supplementary Methods for details.

Genomic analysis

Detailed methods are described in Supplementary Methods. In brief, a whole-genome alignment of all isolates was generated via mapping WGS reads against the chromosomal sequence of NRCS-A isolate CR01 (GenBank accession: LN866849.1) using PHEnix.[17,31] Core-genome single-nucleotide polymorphisms (cgSNPs) were identified from this alignment using snp-sites and counted between isolates as genetic distances using snp-dists.[32,33] A neighbour-joining (NJ) tree was constructed from genetic distances using R package ape[34] to represent the population structure of all *S. capitis* isolates. The 59 NRCS-A-specific marker genes were detected using GeneFinder[35] under requirements for a ≥90% coverage and nucleotide identity to each reference sequence. NRCS-A isolates were confirmed based on isolate clustering in the NJ tree and numbers (> 20) of detected gene markers.

A rooted maximum-likelihood (ML) tree correcting for recombination events was generated from the whole-genome alignment of all NRCS-A isolates using Gubbins to represent the fine population structure of this clone.[36] CgSNPs of NRCS-A isolates were identified in Gubbins's recombination-free alignment of polymorphic sites using snp-sites and counted using snp-dists. Based on the distribution of genetic distances observed in this study (Supplementary Fig. 1), each genetic cluster within the NRCS-A clone

was defined in the ML tree as a high-confidence clade (large bootstrap value of >70% for the root of the clade) comprising ≥5 isolates differed by ≤50 cgSNPs. Ancestral dates of selected subsets of NRCS-A isolates were estimated with BactDating.[37] Isolate metadata were overlaid to the tree and visualised with iTOL.[38] Patient movements were confirmed by clinicians following identification of unique patient identifiers shared between laboratories.

Ethics

National surveillance of communicable diseases and outbreak investigation work at the UKHSA does not require individual patient consent as per Regulation 3 of The Health Service (Control of Patient Information) Regulations 2002.

Data availability

WGS data generated in this study have been deposited in the ENA under the BioProject accession PRJEB51567. Accession numbers and isolate metadata are available in Supplementary Table 1.

Results

Genetic, geographical, and specimen diversities of S. capitis isolates

WGS data were generated from 760 *S. capitis* in this study and combined with published WGS data of 78 isolates. Altogether, we analysed 838 *S. capitis* genomes, consisting of 816 from clinical isolates, 20 from environmental isolates, and two from type strains. Of these 838 isolates, 728 (87%) were collected from infants (< 1 year), 68 (8%) from adults, 20 (2%) from other age groups (1–17 years), and the two type strains had no host-age information. Overall, 86,072 cgSNP sites were found across all isolates when compared to the reference genome CR01, and isolates differed by 0–51,550 (median: 68) cgSNPs pairwise. The NRCS-A clone, which consisted of 750 (90%) isolates, formed a distinct cluster in which isolates differed by 0–632 cgSNPs and carried 23–59 NRCS-A-specific gene markers (Fig. 1 and Supplementary Fig. 2).

In total, 796 out of 838 *S. capitis* isolates were collected from across the UK (Supplementary Fig. 3), comprising 712 NRCS-A isolates and 84 non-NRCS-A isolates, with documented collection dates between March 1999–October 2021 and between January 2006–September 2021, respectively. Of these NRCS-A isolates, 640 (90%) were recovered from infants of ≤90 days of life (584 from normally sterile sites) and 18 were recovered from fomites in two NICUs that were >160 km apart in the South West of England. The number of NRCS-A isolates recovered from normally sterile sites of infants had increased since 2019 (Supplementary Fig. 4), which is in line with clinicians' previous suspicion of increased neonatal *S. capitis* bacteraemia incidence across the UK over this period. NRCS-A isolates were not recovered from airborne particles with settle plates. By contrast, non-NRCS-A isolates were recovered with settle plates in parents/staff spaces in both NICUs where the environmental sampling was conducted (Supplementary Table 1).

In this study, 30 of the 59 NRCS-A-specific gene markers showed ≥95% sensitivity and specificity in detecting NRCS-A isolates (Supplementary Fig. 5), and particularly, nine of these 30 gene markers were present in all NRCS-A isolates, with 97–99% specificity (Supplementary Table 2). Gene *nsr* (100% sensitivity and 97% specificity), which encodes resistance to bacteriocin nisin, was one of these nine conserved genes and the only gene with a confirmed function. Thirteen of the 59 gene markers were unique to the NRCS-A clone (sensitivity: 76–96%), including nine genes (*cas1*, *cas2*, *csm1–6*, and *cas6*) associated with clustered regularly interspaced short palindromic repeats (CRISPR) in the composite SCC*mec*–SCC*cad/ars/cop* cassette.[17] The *tarIJL* gene cluster, which involves in the biosynthesis of wall teichoic acids that contribute to biofilm formation, surface attachment, and resistance to environmental stress,[39] showed a specificity of 95.5% and was present in 99.6% of NRCS-A isolates, including all 18 from fomites. By contrast, this gene cluster was not carried by either non-NRCS-A isolate from air.

Geographical distribution of NRCS-A isolates

NRCS-A isolates were geographically dispersed across the UK. Most of the 712 UK NRCS-A isolates were collected from England (n = 614, 86%), followed by those from Wales (n = 53, 7%), Scotland (n = 40, 6%), and Northern Ireland (n = 5, 1%). The English isolates were obtained from all geographical regions of England: London (n = 235, 38%), West Midlands (n = 125, 20%), South East (n = 73, 12%), South West (n = 68, 11%), Yorkshire and the Humber (n = 33, 5%), East Midlands (n = 24, 4%), North West (n = 23, 4%), East of England (n = 20, 3%), and North East (n = 9, 1%). Four (1%) English isolates did not have regional information.

A large lineage consisting of 611 UK isolates (genetic distances: 0–117 cgSNPs) was identified in the NRCS-A clone (Fig. 2) and was designated NRCS-A_{UK}. These isolates were collected from across the UK in 2005–2021 and comprised of 586 isolates from infants (555 from normally sterile sites), seven blood isolates from children aged 1–5 years old, and 18 isolates from NICU environments. Twenty-eight isolates (all from infants) belonging to a previously identified 'international outbreak' lineage[16] were found within NRCS-A_{UK} (Supplementary Table 1). Further, a sub-lineage (genetic distances: 0–94 cgSNPs) comprising 521 isolates collected in 2011–2021 was identified in NRCS-A_{UK}, including 482 isolates from normally sterile sites of infants (Fig. 2).

Twenty-eight genetic clusters of NRCS-A isolates were defined to further elucidate the population structure of this clone (Fig. 2). Each cluster comprised 5–61 isolates (median: 12), which were collected over 1–6 years except those in cluster N983 (n = 10, collected over 11 years). These 28 genetic clusters consisted of isolates only from the UK and showed a variable distribution within and across geographical regions. First, isolates from nine clusters were found only in single regions and those from the rest of 19 clusters were found in 2–6 regions (Supplementary Fig. 6). Second, isolates from up to six clusters were identified in two regions in 36 instances (Supplementary Fig. 7). Moreover, four genetic clusters were present across Wales and England, whereas one cluster was distributed across Scotland and England, and one across Scotland and Northern Ireland (Supplementary Fig. 6). Notably, the number of identified genetic clusters in each geographical region was positively correlated (two-sided p-value = 0.0014) with the number of NRCS-A isolates voluntarily referred from this region (Supplementary Fig. 8). Thus, greater numbers of genetic clusters were found in London, the South East, South West, West Midlands, and East of England (6–12 clusters per region).

Genetic diversity of NRCS-A isolates from the same patients

NRCS-A isolates collected from the same patients were compared to assess within-host diversity and genetic variation over time. In total, eight pairs of same-day isolates were recovered from eight infants, and each pair of isolates were genetically identical (genetic distance: 0 cgSNP). In addition, 65 sequential NRCS-A isolates were obtained from 30 infants (2–5 isolates per infant) in 1–89 days. Sequential isolates from the same infants differed by ≤5 cgSNPs (median: 2 cgSNPs) except 11 isolates collected from Infants P001, P019, P022, and P030 in 4–81 days, which differed by 8–196 cgSNPs (Fig. 3 and Supplementary Table 3).

All four blood isolates sequentially obtained from P001 in November–December 2013 belonged to the 'international outbreak'

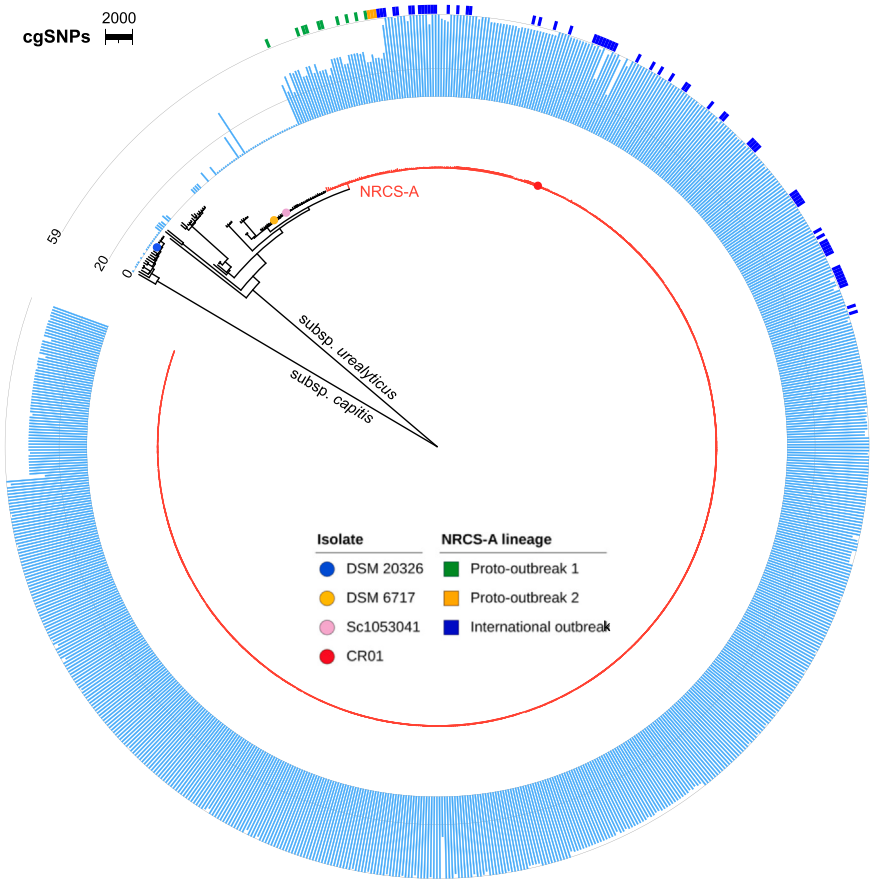

Fig. 1. Population structure of 838 *S. capitis* isolates and the number of NRCS-A-specific gene markers detected in each isolate. A midpoint-rooted neighbour-joining tree was reconstructed from genetic distances between 838 *S. capitis* isolates. The NRCS-A clone (750 isolates) is highlighted by red branches. The bar plot (blue) illustrates the number of NRCS-A-specific gene markers detected in each isolate, and the outmost colour ring represents NRCS-A lineages previously identified in published genomes by Wirth et al.[16] The genome of non-NRCS-A isolate Sc1053041 was used as an outgroup to root the maximum-likelihood phylogenetic tree in Fig. 2. The chromosomal sequence of CR01 is the reference used for read mapping, and *S. capitis* type strains DSM 20326 (subsp. *capitis*) and DSM 6717 (subsp. *urealyticus*) were used for subspecies identification.

lineage and appeared to be the same strain (genetic distances: 0–1 cgSNP), whereas a nose-swab isolate collected from P001 in February 2014 belonged to the 'proto-outbreak 1' lineage and differed from the four blood isolates by 195–196 cgSNPs (Supplementary Table 4). This genetic divergence indicates distinct infection and carriage strains in this infant. Conversely, blood and nose-swab isolates collected from Infant P042 in two days differed by one cgSNP, suggesting an infection and carriage of the same strain. Furthermore, the relatively large genetic distances (8–40 cgSNPs) between paired sequential isolates recovered from blood or a central venous catheter of Infants P019, P022, and P030 in 4–13 days (Supplementary Table 3) imply multi-strain bacteraemia episodes.

Genetic links between clinical and fomite NRCS-A isolates in a NICU

We investigated within-hospital genetic relatedness between clinical and fomite isolates. Of NRCS-A isolates identified in this study, only five out of the 18 fomite isolates had clinical isolates (n = 32) matched by the same hospital (Hospital L18). These 37 isolates comprised 31 clinical isolates (26 from blood, four from vascular catheters, and one from an endotracheal tube) from infants in this hospital's NICU, one pleural-fluid isolate from an adult, and five fomite isolates from internal and external surfaces of baby incubators. Three of the 28 genetic clusters were identified in these 37 isolates (Fig. 4). The five fomite isolates were interspersed with five blood isolates (from four infants in the same NICU) in a distinct clade where isolates (n = 10) differed by 0–5 cgSNPs (Supplementary Table 5), suggesting contamination and infection of the same strain. Moreover, eight isolates of this clade were collected within 45 days (late July–early September 2021), including four genetically identical isolates that were recovered from two clinical specimens (Infants P014 and P190), a stethoscope in Infant P014's cot space, and an oxygen control, respectively (Supplementary Table 5). Nevertheless, directions of transmission between infants and fomites within this NICU could not be inferred for this NRCS-A strain with the 10 isolates recovered owing to uncertainty in phylogenetic relatedness between these isolates (low bootstrap values: 7–31%) and a lack of longitudinal environmental isolates.

Inter-hospital genetic links between NRCS-A isolates

Genetic links between NRCS-A isolates were identified across two hospitals in the same geographical region (Wales) and were associated with inter-hospital infant movements. In July 2021, Infant P003 (with bacteraemia) admitted to the NICU of Hospital L11 and then was transferred into an NNU of Hospital L40. Three blood isolates were recovered from P003 within nine days in these two hospitals and formed a distinct clade with a blood isolate recovered from Infant P180 in early-August in Hospital L40 (Fig. 5a). These four isolates differed by 0–1 cgSNPs, indicating presence of the same strain in both infants. However, we could not conclude the plausible transmission of this strain from P003 to P180 due to a lack of clinical details and uncertainty in phylogenetic relatedness between these four isolates (low bootstrap values: 30–32%).

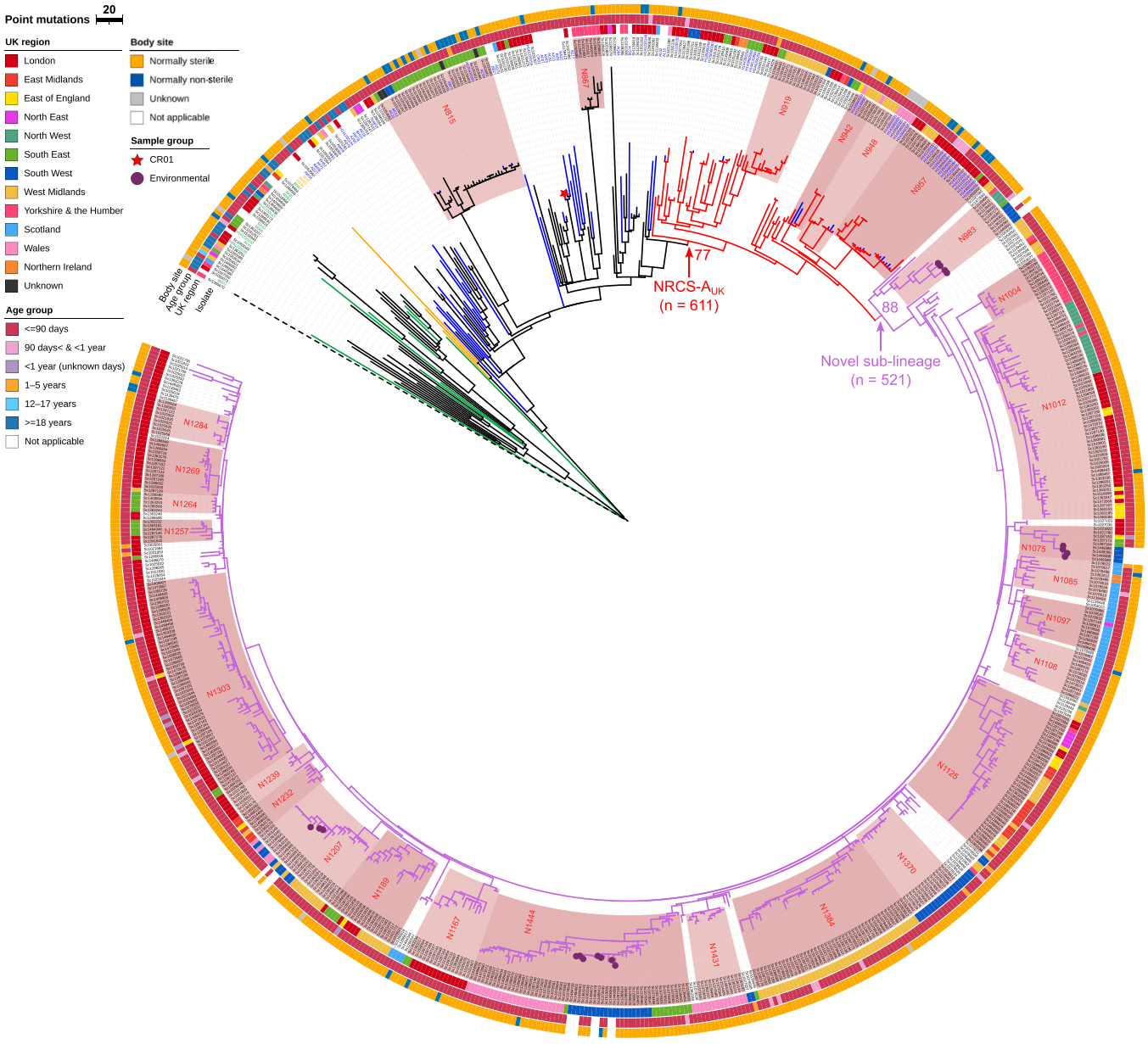

Fig. 2. Recombination-corrected maximum-likelihood tree of 750 NRCS-A isolates collected between 1997 and October 2021 from 19 countries. This tree is rooted on a closely related non-NRCS-A isolate Sc1053041 (this study, not shown) determined in the neighbour-joining tree for all 838 isolates (Fig. 1). Each genetic cluster is indicated by a pink shade and labelled (red) by its root branch. White gaps in the coloured ring 'UK region' indicate 38 international isolates. Red branches indicate the possible NRCS-A$_{UK}$ lineage including its novel sub-lineage comprising UK isolates collected in 2011–2021 (purple branches). Bootstrap values are labelled beside root branches of both lineages. Green, orange, and blue branches represent the international 'proto-outbreak 1', 'proto-outbreak 2' and 'outbreak' lineages identified by Wirth et al., respectively.[16] The scale bar denotes 20 recombination-free point mutations per unit branch length. The dashed branch representing the NRCS-A isolate Sc1365670 has been arbitrarily shortened from its original length of 531 point mutations to 355 to preserve details of short branches in the figure.

Movement-associated genetic links were also identified between NRCS-A isolates from hospitals in different geographical regions. Specifically, two genetically identical blood isolates were recovered within 90 days in June–September 2021 from Infant P018 (with bacteraemia), who was transferred from a NICU in London (Hospital L15) to a NICU in the East of England (Hospital L10) in late-August. Both isolates differed from five closely related clinical isolates (collected from blood or cerebrospinal fluid of four infants admitted to L15 in April–September 2021) by 2–6 cgSNPs and formed a unique cluster with these five isolates (Fig. 5b), suggesting an invasive strain that might have circulated in the NICU of L15 and was introduced into the NICU of L10 via the transfer of Infant P018. Nonetheless, this strain might not be able to persist in L10 because no subsequent isolates of this strain were referred from this hospital to the UKHSA Reference Laboratory by the end of October 2021.

Discussion

Based on WGS data, this study reveals that the *S. capitis* NRCS-A clone has spread among NNUs across all geographical regions in the UK with the first documented appearance in 1999. We discovered that 30 of the 59 previously reported NRCS-A-specific gene markers were adequate (≥95% sensitivity and specificity) for distinguishing the NRCS-A clone from the others in our genome collection. Twenty-eight phylogenetically distinct genetic clusters were identified within the NRCS-A clone (with isolates in each cluster collected within 11 years) in the UK, and isolates

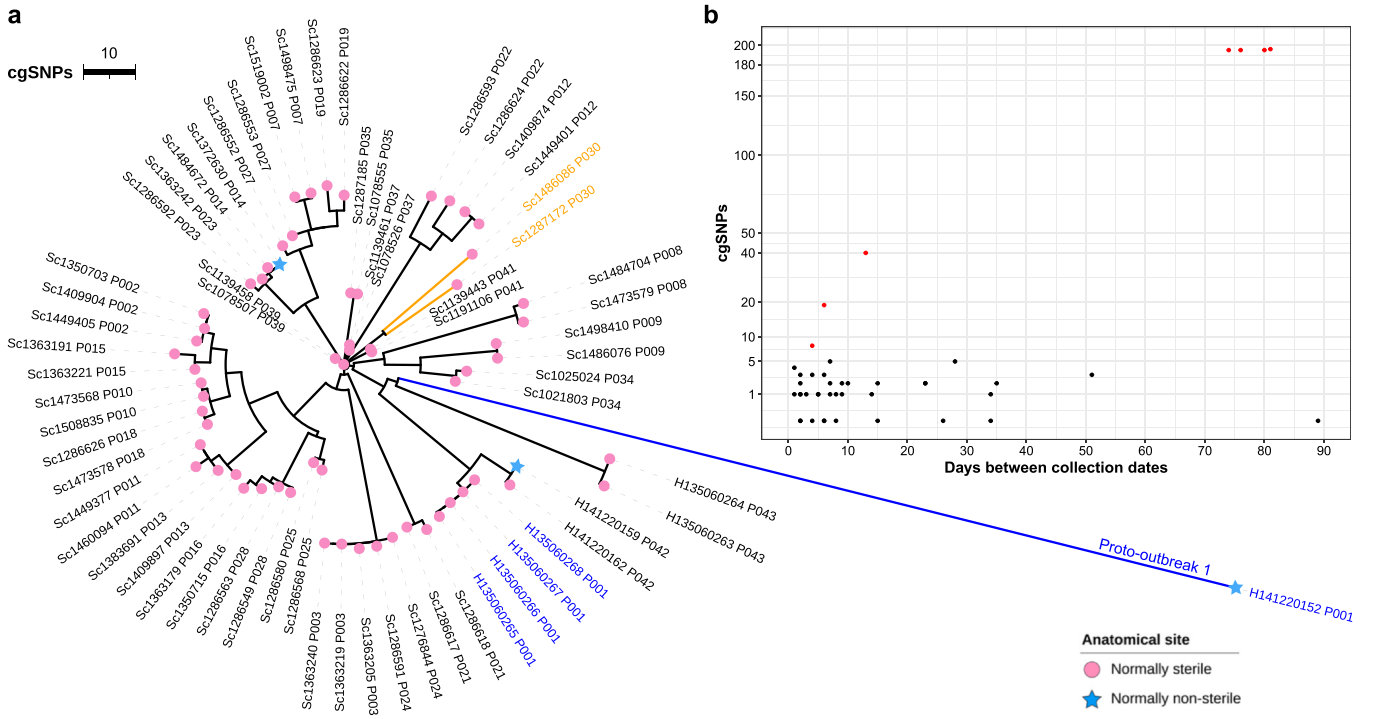

Fig. 3. Genetic divergence between sequential NRCS-A isolates from the same patients. (**a**) An unrooted neighbour-joining tree illustrating genetic distances between 65 sequential isolates from 30 infants. Highlighted branches and tip labels represent same-patient isolates (Infant P001, blue; and P030, gold) that differed by a maximum of 196 or 40 cgSNPs. (**b**) A scatter plot comparing genetic distances with respect to days between collection dates of isolates from the same patient. Outliers of genetic distances are highlighted in red. Abbreviation: cgSNP, core-genome single-nucleotide polymorphism.

from 19 genetic clusters were found in 2–6 geographical regions, suggesting diversification of the NRCS-A clone followed by inter-regional dispersion, although mechanisms of transmission are yet to be elucidated. Clinical and fomite NRCS-A isolates from a NICU were closely related within a unique phylogenetic cluster amongst diverse clinical isolates from the same NICU. Further, this study discovers inter-hospital genetic links between NRCS-A isolates related to intra-/inter-regional infant movements, implying the potential of such movements to spread the NRCS-A clone.

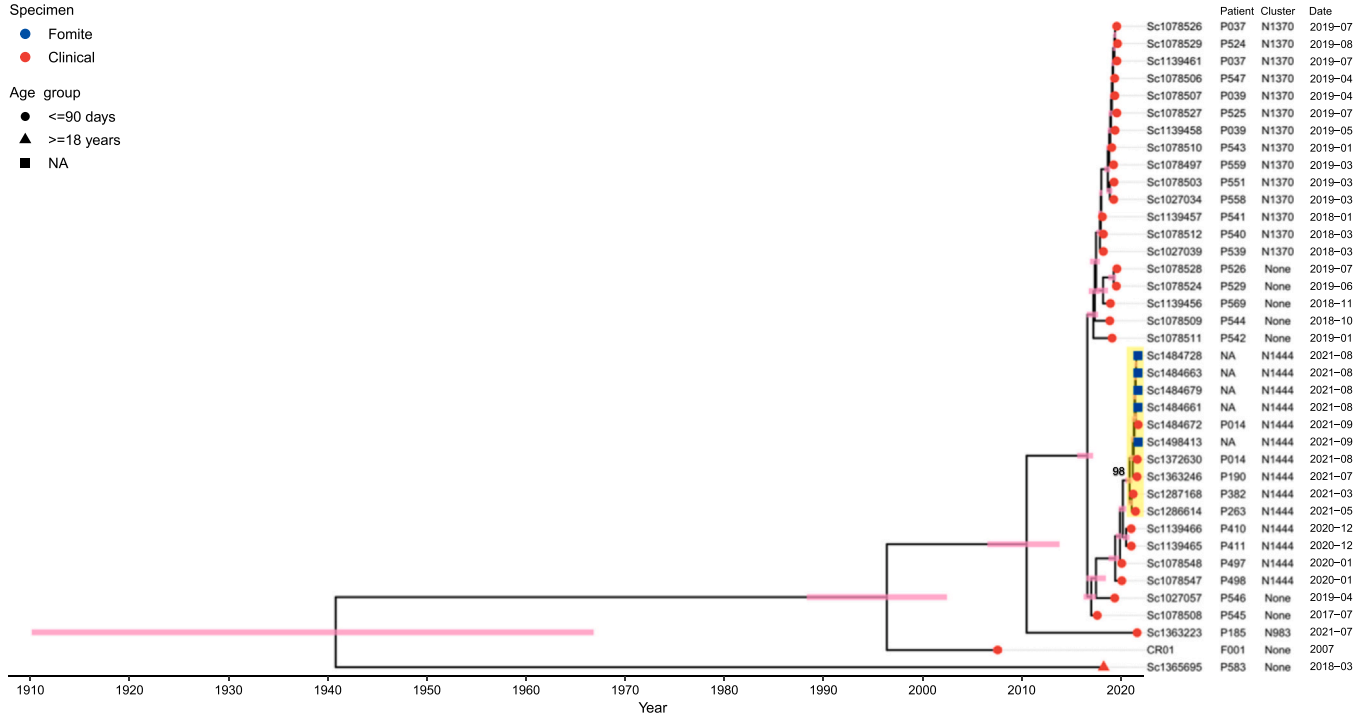

Fig. 4. Phylogeny of 37 NRCS-A isolates from Hospital L18. Error bars (pink) indicate the range of possible isolate-collection dates (when the explicit dates were not available) and the 95% confidence intervals of ancestral dates. The chromosomal sequence of isolate CR01 was used as the reference for comparison, and genetic clusters of isolates are the same as those defined in Fig. 2. The bootstrap value supporting the highlighted clade (light yellow) is noted above the root branch of this clade. Abbreviation: NA, not applicable.

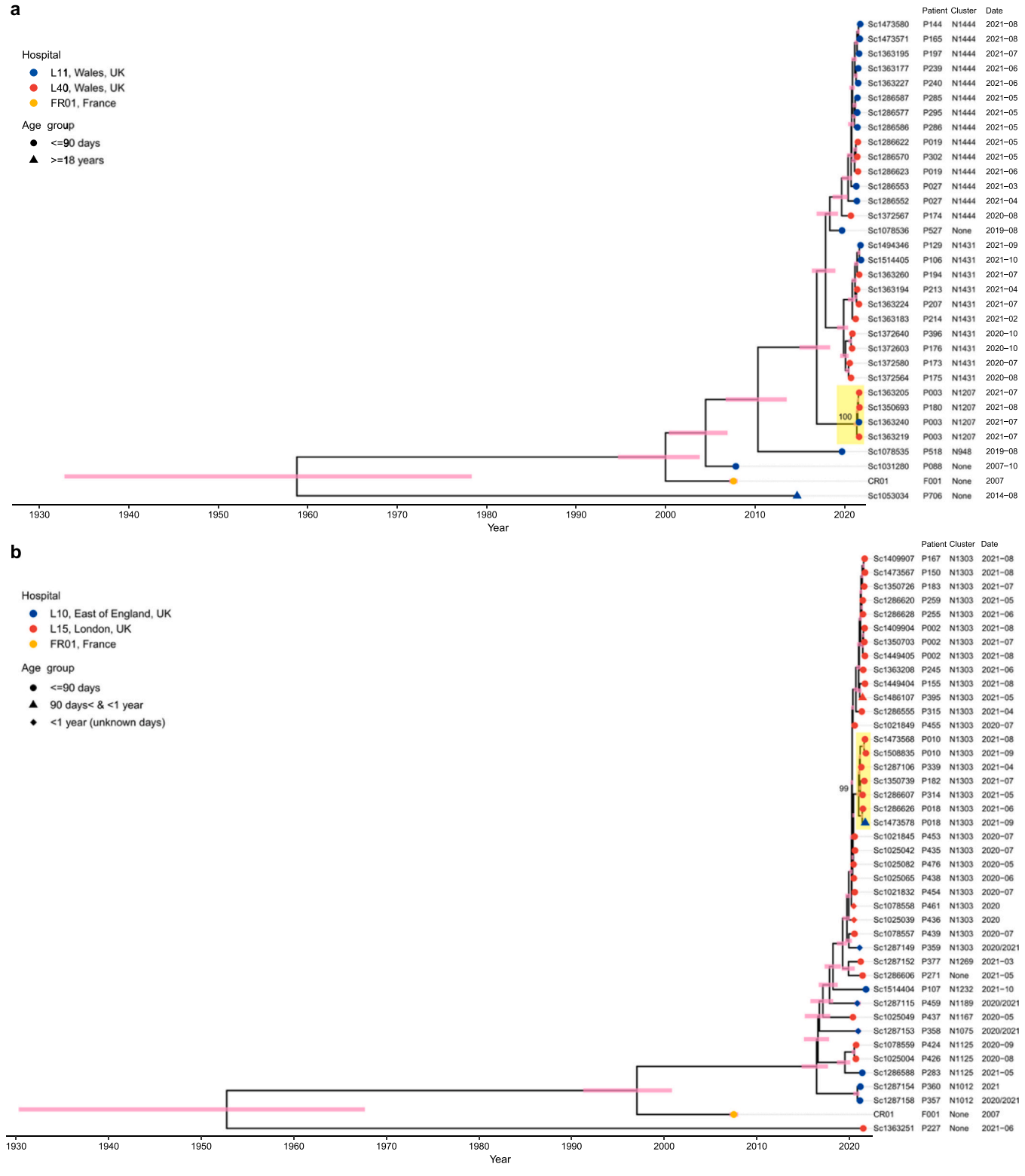

Fig. 5. Phylogeny of NRCS-A isolates from four UK hospitals. Error bars (pink) indicate the range of possible isolate-collection dates (when the explicit dates were not available) and the 95% confidence intervals of ancestral dates. The chromosomal sequence of French clinical isolate CR01 was used as the reference for sequence comparison, and genetic clusters of isolates are the same as those defined in Fig. 2. The bootstrap value of each highlighted clade (light yellow) is noted above the root branch of this clade. (**a**) Isolates from two hospitals that are approximately 18 km apart in two cities in Wales. (**b**) Isolates from two hospitals that are approximately 80 km apart in London and the East of England. Abbreviation: NA, not applicable.

While the public health relevance and genetic features of the NRCS-A clone are further investigated, our study adds to knowledge about nationwide presence of this clone in NNUs, which has been reported in Western Europe and Australia.[12,19] With the unprecedented amount of *S. capitis* WGS data generated by the UKHSA, we have reconstructed the population structure of the NRCS-A clone across all geographical regions in the UK and discovered a possible UK-specific NRCS-A lineage (NRCS-A_{UK}) that

might originate from the previously described international outbreak lineage and differs from the two proto-outbreak lineages.[16] Further research is needed to elucidate the transmission, adaptation, and evolution of the NRCS-A clone in the UK.

Our report has two clinical implications for the prevention and treatment of *S. capitis* infection in NNUs. First, since the NRCS-A clone rapidly adapts to vancomycin treatment,[40] the identification of the UK-wide spread of this clone has informed the UKHSA to recommend optimising neonatal vancomycin dosing.[41] Second, the identification of genetic links between fomite and clinical NRCS-A isolates in a NICU suggests an opportunity to improve decontamination practice, albeit endemic reservoirs of the NRCS-A clone resulting from colonisation on ineffectively disinfected surfaces of medical equipment in NNUs remain to be confirmed.[23,42] Additionally, presence of the biofilm-associated *tarIJL* gene cluster in all NRCS-A fomite isolates and absence of NRCS-A isolates from sampled airborne particles in two distinct NICUs imply that contact of contaminated surfaces would play a predominant role in disseminating the NRCS-A clone. Therefore, it is necessary to study the persistence of the NRCS-A clone on surfaces in NNUs and assess the effectiveness of existing decontamination procedures to prevent transmission of this clone.[20,43]

Our study has limitations. First, since we only sought presumptive NRCS-A isolates with specific antibiograms, non-NRCS-A isolates and possibly, NRCS-A isolates of unexpected antibiograms, are underrepresented in our collection. Second, the referral of isolates was at the discretion of microbiology laboratories and depended on isolate availability, which might have contributed to the geographical bias in the sample size and missed some NRCS-A genetic clusters in insufficiently sampled regions. Third, we did not have adequate clinical information to discern invasive isolates or contamination for clinical relevance, nor had epidemiological information to infer transmission events or determine to what extent the geographical network of NRCS-A genetic clusters matched the hospital network for infant transfer. Fourth, systematic screening of infants for *S. capitis* carriage, same-case multi-colonial sampling,[44] and continuous environmental sampling were not conducted to generate *S. capitis* sequencing data that enable the estimation of the intra-NNU genetic diversity, which is pivotal for the inference of endogenous infections and transmission events.[45]

Despite above limitations, the dataset of nationwide *S. capitis* genomes generated in this study from UKHSA's culture collection is an outstanding resource for future research and integrative applications such as hospital and international surveillance of pathogen genomes.[46,47] We report close genetic relatedness between clinical and environmental *S. capitis* isolates from the same NICU, which has addressed an understudied aspect of *S. capitis* genomic epidemiology. Further, the wide spread and genetic diversity of the NRCS-A clone revealed by our work indicate the need for typing methods for *S. capitis*, including WGS-based approaches to genomic surveillance and scalable genetic tools (*e.g.*, multi-locus sequence typing and NRCS-A-specific polymerase chain reaction) for resource-challenged settings where WGS is not easily accessible.

In conclusion, the UKHSA IMT has bought together the national reference laboratory, microbiology laboratories, and clinicians from across the UK and conducted large-scale WGS of *S. capitis* isolates. With the extensive genomic data generated, our study confirms the wide spread of the *S. capitis* NRCS-A clone among NNUs across the UK and urges further genomic, epidemiological, and clinical research on this microorganism.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.06.020.

References

1. O'Sullivan Julie N, O'Connor Paula M, Rea Mary C, O'Sullivan Orla, Walsh Calum J, Healy Brian, et al. *Nisin J, a novel natural nisin variant, is produced by Staphylococcus capitis sourced from the human skin microbiota. J Bacteriol 2020* ;**202**(3). https://doi.org/10.1128/JB.00639-19. e00639-19.
2. Hariharan Harry, Matthew Vanessa, Fountain Jacqueline, Snell Alicia, Doherty Devin, King Brittany, et al. *Aerobic bacteria from mucous membranes, ear canals, and skin wounds of feral cats in Grenada, and the antimicrobial drug susceptibility of major isolates. Comp Immunol Microbiol Infect Dis 2011* ;**34**(2):129–34. https://doi.org/10.1016/j.cimid.2010.05.001
3. Cantas Leon, Sørby Jan Roger Torp, Aleström Peter, Sørum Henning. *Culturable gut microbiota diversity in zebrafish. Zebrafish 2012* ;**9**(1):26–37. https://doi.org/10.1089/zeb.2011.0712
4. Golińska Edyta, Strus Magdalena, Tomusiak-Plebanek Anna, Więcek Grażyna, Kozień Łucja, Lauterbach Ryszard, et al. *Coagulase-negative staphylococci contained in gut microbiota as a primary source of sepsis in low- and very low birth weight neonates. J Clin Med 2020* ;**9**(8):2517. https://doi.org/10.3390/jcm9082517
5. Rasigade Jean-Philippe, Raulin Olivia, Picaud Jean-Charles, Tellini Charlotte, Bes Michele, Grando Jacqueline, et al. *Methicillin-resistant Staphylococcus capitis with reduced vancomycin susceptibility causes late-onset sepsis in intensive care neonates. PLOS ONE 2012* ;**7**(2):e31548.
6. Cui Bintao, Smooker Peter M, Rouch Duncan A, Daley Andrew J, Deighton Margaret A. *Differences between two clinical Staphylococcus capitis subspecies as revealed by biofilm, antibiotic resistance, and pulsed-field gel electrophoresis profiling. J Clin Microbiol 2013* ;**51**(1):9–14. https://doi.org/10.1128/jcm.05124-11
7. Azimi T, Mirzadeh M, Sabour S, Nasser A, Fallah F, Pourmand MR. *Coagulase-negative staphylococci (CoNS) meningitis: a narrative review of the literature from 2000 to 2020. New Microbes New Infect 2020* ;**37**:100755. https://doi.org/10.1016/j.nmni.2020.100755
8. Heath Victoria, Cloutman-Green Elaine, Watkin Samuel, Karlikowska Magdalena, Ready Derren, Hatcher James, et al. *Staphylococcus capitis: review of its role in infections and outbreaks. Antibiotics 2023* ;**12**(4):669. https://doi.org/10.3390/antibiotics12040669
9. Natsis Nicola E, Cohen Philip R. *Coagulase-negative Staphylococcus skin and soft tissue infections. Am J Clin Dermatol 2018* ;**19**(5):671–7. https://doi.org/10.1007/s40257-018-0362-9
10. Tevell Staffan, Baig Sharmin, Hellmark Bengt, Martins Simoes Patricia, Wirth Thierry, Butin Marine, et al. *Presence of the neonatal Staphylococcus capitis outbreak clone (NRCS-A) in prosthetic joint infections. Sci Rep 2020* ;**10**(1):22389. https://doi.org/10.1038/s41598-020-79225-x
11. Paranthaman Karthik, Wilson Allegra, Verlander Neville, Rooney Graeme, Macdonald Neil, Nsonwu Olisaeloka, et al. *Trends in coagulase negative staphylococci (CoNS), England, 2010-2021. Access Microbiol 2023* ;**5**(6). https://doi.org/10.1099/acmi.0.000491.v3. 000491.v3.
12. Decalonne Marie, Dos Santos Sandra, Gimenes Rémi, Goube Florent, Abadie Géraldine, Aberrane Saïd, et al. *Staphylococcus capitis isolated from bloodstream infections: a nationwide 3-month survey in 38 neonatal intensive care units. Eur J Clin Microbiol Infect Dis 2020* ;**39**(11):2185–94. https://doi.org/10.1007/s10096-020-03925-5
13. Lamers Ryan P, Gowrishankar Muthukrishnan, Castoe Todd A, Tafur Sergio, Cole Alexander M, Parkinson Christopher L. *Phylogenetic relationships among Staphylococcus species and refinement of cluster groups based on multilocus data. BMC Evolut Biol 2012* ;**12**(1):171. https://doi.org/10.1186/1471-2148-12-171
14. Bannerman Tammy L, Kloos Wesley E. *Staphylococcus capitis subsp. ureolyticus subsp. nov. from Human Skin. Int J Syst Evolut Microbiol 1991* ;**41**(1):144–7. https://doi.org/10.1099/00207713-41-1-144
15. Chong Charlotte E, Bengtsson Rebecca J, Horsburgh Malcolm James. *Comparative genomics of Staphylococcus capitis reveals species determinants. Front Microbiol 2022* ;**13**:1005949. https://doi.org/10.3389/fmicb.2022.1005949
16. Wirth Thierry, Bergot Marine, Jean-Philippe Rasigade, Pichon Bruno, Barbier Maxime, Martins-Simoes Patricia, et al. *Niche specialization and spread of Staphylococcus capitis involved in neonatal sepsis. Nat Microbiol 2020* ;**5**(5):735–45. https://doi.org/10.1038/s41564-020-0676-2
17. Martins Simões P, Lemriss H, Dumont Y, Lemriss S, Rasigade JP, Assant-Trouillet S, et al. *Single-molecule sequencing (PacBio) of the Staphylococcus capitis NRCS-A clone reveals the basis of multidrug resistance and adaptation to the neonatal intensive care unit environment. Front Microbiol 2016* ;**7**:1991. https://doi.org/10.3389/fmicb.2016.01991
18. Stenmark Bianca, Hellmark Bengt, Söderquist Bo. *Genomic analysis of Staphylococcus capitis isolated from blood cultures in neonates at a neonatal intensive care unit in Sweden. Eur J Clin Microbiol Infect Dis Publ Eur Soc Clin Microbiol 2019* ;**38**(11):2069–75. https://doi.org/10.1007/s10096-019-03647-3
19. Butin M, Rasigade J-P, Martins-Simões P, Meugnier H, Lemriss H, Goering RV, et al. *Wide geographical dissemination of the multiresistant Staphylococcus capitis NRCS-A clone in neonatal intensive-care units. Clin Microbiol Infect 2016* ;**22**(1):46–52. https://doi.org/10.1016/j.cmi.2015.09.008
20. Ory Jérôme, Cazaban Michel, Richaud-Morel Brigitte, Di Maio Massimo, Dunyach-Remy Catherine, Pantel Alix, et al. *Successful implementation of infection control measure in a neonatal intensive care unit to combat the spread of pathogenic multidrug resistant Staphylococcus capitis. Antimicrob Resist Infect Control 2019* ;**8**:57. https://doi.org/10.1186/s13756-019-0512-8
21. Qu Yue, Li Yali, Cameron David R, Easton Christopher D, Zhu Xuebo, Zhu Minli, et al. *Hyperosmotic infusion and oxidized surfaces are essential for biofilm formation of Staphylococcus capitis from the neonatal intensive care unit. Front Microbiol 2020* ;**11**:920. https://doi.org/10.3389/fmicb.2020.00920
22. Chavignon Marie, Coignet Ludivine, Bonhomme Mélanie, Bergot Marine, Tristan Anne, Verhoeven Paul, et al. *Environmental persistence of Staphylococcus capitis NRCS-A in neonatal intensive care units: role of biofilm formation, desiccation, and disinfectant tolerance. Microbiol Spectr 2022* ;**10**(6):e04215–22. https://doi.org/10.1128/spectrum.04215-22
23. Butin Marine, Dumont Yann, Monteix Alice, Raphard Aurane, Roques Christine, Martins Simoes Patricia, et al. *Sources and reservoirs of Staphylococcus capitis NRCS-A inside a NICU. Antimicrob Resist Infect Control 2019* ;**8**(1):157. https://doi.org/10.1186/s13756-019-0616-1
24. Public Health England. *Increased incidence of detections of Staphylococcus capitis in neonatal clinical samples (PHE Briefing Note 2021/005)*; 2021.
25. UK Health Security Agency. *Increased detection of Staphylococcus capitis in samples from hospitalised infants (update) (UKHSA Briefing Note 2021/078)*; 2021.
26. Leinonen Rasko, Akhtar Ruth, Birney Ewan, Bower Lawrence, Cerdeno-Tárraga Ana, Cheng Ying, et al. *The European nucleotide archive. Nucleic Acids Res 2011* ;**39**(Suppl. 1):D28–31. https://doi.org/10.1093/nar/gkq967
27. Bolger Anthony M, Lohse Marc, Usadel Bjoern. *Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014* ;**30**(15):2114–20. https://doi.org/10.1093/bioinformatics/btu170
28. Shen Wei, Le Shuai, Li Yan, Hu Fuquan. *SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. PLOS ONE 2016* ;**11**(10):e0163962. https://doi.org/10.1371/journal.pone.0163962
29. Wood Derrick E, Lu Jennifer, Langmead Ben. *Improved metagenomic analysis with Kraken 2. Genome Biol 2019* ;**20**(1):257. https://doi.org/10.1186/s13059-019-1891-0
30. Lu Jennifer, Breitwieser Florian P, Thielen Peter, Salzberg Steven L. *Bracken: estimating species abundance in metagenomics data. PeerJ Comput Sci 2017* ;**2017**(1):e104. https://doi.org/10.7717/peerj-cs.104
31. UKHSA. *PHEnix*; n.d. Available at: ⟨https://github.com/phe-bioinformatics/PHEnix⟩.
32. Page Andrew J, Taylor Ben, Delaney Aidan J, Soares Jorge, Seemann Torsten, Keane Jacqueline A, et al. *SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments e000056–e000056 . Microb Genom 2016* ;**2**(4):e000056. https://doi.org/10.1099/mgen.0.000056
33. Torsten Seemann, Fabian Klötzl, Andrew J. Page, *snp-dists*; n.d. Available at: ⟨https://github.com/tseemann/snp-dists⟩.
34. Paradis E, Claude J, Strimmer K. *APE: analyses of phylogenetics and evolution in R language. Bioinformatics 2004* ;**20**(2):289–90. https://doi.org/10.1093/bioinformatics/btg412
35. UKHSA. *GeneFinder*; n.d. Available at: ⟨https://github.com/phe-bioinformatics/gene_finder⟩.
36. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. *Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res 2014* ;**43**(3):e15. https://doi.org/10.1093/nar/gku1196
37. Didelot X, Croucher NJ, Bentley SD, Harris SR, Wilson DJ. *Bayesian inference of ancestral dates on bacterial phylogenetic trees. Nucleic Acids Res 2018* ;**46**(22):e134. https://doi.org/10.1093/nar/gky783
38. Letunic Ivica, Bork Peer. *Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 2021* ;**49**(W1):W293–6. https://doi.org/10.1093/nar/gkab301
39. Xia Guoqing, Kohler Thomas, Peschel Andreas. *The wall teichoic acid and lipoteichoic acid polymers of Staphylococcus aureus. Int J Med Microbiol 2010* ;**300**(2):148–54. https://doi.org/10.1016/j.ijmm.2009.10.001
40. Butin M, Martins-Simões P, Picaud JC, Kearns A, Claris O, Vandenesch F, et al. *Adaptation to vancomycin pressure of multiresistant Staphylococcus capitis NRCS-A involved in neonatal sepsis. J Antimicrob Chemother 2015* ;**70**(11):3027–31. https://doi.org/10.1093/jac/dkv217

41. Harvey EJ, Ashiru-Oredope D, Hill LF, Demirjian A, Jauneikaite E, Wan Y, et al. *Need for standardized vancomycin dosing for coagulase-negative staphylococci in hospitalized infants. Clin Microbiol Infect 2023* ;**29**(1):10–2. https://doi.org/10.1016/j.cmi.2022.09.016
42. Carter GP, Ussher JE, Da Silva AG, Baines SL, Heffernan H, Riley TV, et al. *Genomic analysis of multiresistant Staphylococcus capitis associated with neonatal sepsis. Antimicrob Agents Chemother 2018* ;**62**(11). https://doi.org/10.1128/AAC.00898-18. e00898-18.
43. UK Health Security Agency, Good *IPC practice for the cleaning and handling of incubators and other equipment in neonatal units*. Available at ⟨https://www.gov.uk/government/publications/infection-prevention-and-control-incubators-on-neonatal-units/good-ipc-practice-for-the-cleaning-and-handling-of-incubators-and-other-equipment-in-neonatal-units⟩. [Accessed 9 May 2022].
44. Hall MD, Holden MTG, Srisomang P, Mahavanakul W, Wuthiekanun V, Limmathurotsakul D, et al. *Improved characterisation of MRSA transmission using within-host bacterial sequence diversity. eLife 2019* ;**8**:e46402. https://doi.org/10.7554/eLife.46402
45. Coll F, Raven KE, Knight GM, Blane B, Harrison EM, Leek D, et al. *Definition of a genetic relatedness cutoff to exclude recent transmission of meticillin-resistant Staphylococcus aureus: a genomic epidemiology analysis. Lancet Microbe 2020* ;**1**(8):e328–35. https://doi.org/10.1016/S2666-5247(20)30149-X
46. Werner G, Couto N, Feil EJ, Novais A, Hegstad K, Howden BP, et al. *Taking hospital pathogen surveillance to the next level. Microb Genom 2023* ;**9**(4):001008. https://doi.org/10.1099/mgen.0.001008
47. Carey ME, Dyson ZA, Argimón S, Cerdeira L, Yeats C, Aanensen D, et al. *Unlocking the potential of genomic data to inform typhoid fever control policy: supportive resources for genomic data generation, analysis, and visualization. Open Forum Infect Dis 2023* ;**10**(Suppl. 1):S38–46. https://doi.org/10.1093/ofid/ofad044