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The detection, survival and persistence of *Staphylococcus capitis* NRCS-A in neonatal units in England.

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Summary

Background: The multi-drug resistant *Staphylococcus capitis* clone, NRCS-A is increasingly associated with late-onset sepsis in low birthweight newborns in neonatal intensive care units (NICUs) in England and globally. Understanding where this bacterium survives and persists within the NICU environment is key to developing and implementing effective control measures.

Aim: To investigate the potential for *S. capitis* to colonise surfaces within NICUs.

Methods: Surface swabs were collected from four NICUs with and without known NRCS-A colonisations/infections present at the time of sampling. Samples were cultured and *S. capitis* isolates analysed via whole genome sequencing. Survival of NRCS-A on plastic surfaces was assessed over time and compared to that of non-NRCS-A isolates. The bactericidal activity of commonly used chemical disinfectants against *S. capitis* was assessed.

Findings: Of 173 surfaces sampled, 40 (21.1%) harboured *S. capitis* with 30 isolates (75%) being NRCS-A. Whilst *S. capitis* was recovered from surfaces across the NICU, the NRCS-A clone was rarely recovered from outside the immediate neonatal bedspace. Incubators and other bedside equipment were contaminated with NRCS-A regardless of clinical case detection. In the absence of cleaning, *S. capitis* was able to survive for 3 days with minimal losses in viability ($< 0.5 \log_{10}$ reduction). Sodium troclosene and a QAC-based detergent/disinfectant reduced *S. capitis* to below detectable levels.

Conclusion: *S. capitis* NRCS-A can be readily recovered from the NICU environment, even in units with no recent reported clinical cases of *S. capitis* infection, highlighting a need for appropriate national guidance on cleaning within the neonatal care environment.

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Introduction

Late-onset neonatal sepsis is a common cause of morbidity and mortality in hospitalised infants, particularly those born pre-term. Empiric treatment usually combines a beta-lactam antibiotic with an aminoglycoside (e.g. gentamicin). However, increasing antibiotic resistance among the coagulase-negative staphylococci, the most frequent cause of late-onset sepsis, has led to alternative antibiotics (e.g. vancomycin) increasingly being used [1].

Staphylococcus capitis NRCS-A has been detected in NICUs worldwide [2] and is a leading cause of neonatal sepsis [3]. NRCS-A isolates exhibit resistance to beta-lactams and aminoglycosides and reduced susceptibility to vancomycin has been noted. The ability of bacteria to adapt to the presence of antimicrobials can provide a selective advantage and it is hypothesised that the emergence and wide geographical dissemination of the NRCS-A clone has been driven by antibiotic selective pressures specific to the NICU setting [4].

Once present within a NICU, the NRCS-A clone can continue to circulate [3, 5]. The exact reasons for this are not yet known. Previous studies suggest that there is no chronic carriage of caregivers [6, 7] and whilst its ability to attach, and to contaminate a variety of environmental surfaces has been reported, no single environmental niche has been identified [6-8].

During summer 2020, the UK Health Security Agency was alerted to a perceived increase in invasive infections caused by *S. capitis* in hospitalised infants. A national incident was declared in June 2021 and, as part of this response, an investigation focusing on the healthcare environment was carried out - specifically the potential for *S. capitis* to colonise and to persist on surfaces within NICUs.

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Methods

Field Study

Study setting

Environmental sampling was carried out in four NICUs located in the south west of England. One NICU was visited on two separate occasions and two had previously reported clinical detection(s) of the *S. capitis* NRCS-A clone. Of the five sampling occasions, two were carried out when a neonate known to be colonised with *S. capitis* NRCS-A was present on the unit.

In all four NICUs, healthcare staff carried out daily or twice-daily damp-dusting using hot soapy water, detergent wipes, QAC-based detergent/disinfectant wipes or a sodium troclosene solution (1000 ppm available chlorine). Whilst protocols differed by unit, in all cases, incubators were changed every 7 days, or earlier. Between babies, the incubator was dismantled and cleaned using a detergent and a

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sodium troclosene solution (1000 ppm available chlorine). Terminal cleaning and disinfection was carried out by the local domestic team, NICU technical support workers or nurses.

Environmental sampling

A zoning assessment, similar to those carried out previously [9], was used to identify potential reservoirs of *S. capitis*. Theoretical environmental 'zones' (Figure 1) encompassed spaces occupied by individual neonates (zone 1) and incorporated the internal surfaces of the incubator (including dedicated stethoscope; zone 1A), surfaces associated with the immediate bedspace (including the external surfaces of the incubator; Zone 1B) and the wider NICU bay (zone 1C). Environmental samples were also collected in areas used by families (zone 2) and from staff only spaces (zone 3). On four sampling occasions, a neonatal incubator had been removed from the bay for cleaning. This provided an opportunity to take additional samples from within Zone 1A and, on one occasion, to assess the efficacy of terminal disinfection.

All (non-porous) surfaces were sampled using nylon flocked swabs wetted with liquid amies solution (ESwab®; Copan Diagnostics Inc, California, USA). Swabs were snapped into 1 mL liquid amies and vortexed to release bacteria from the swab bud. Aliquots (100 µL) of the resulting suspension were cultured on Columbia Agar with 5% horse blood (CBA; E & O Laboratories Ltd, Bonnybridge, UK). Porous surfaces (e.g. incubator covers) were sampled using agar contact plates. Passive air sampling was performed within each zone by exposing CBA settle plates to the air for 2 h. The ambient temperature and relative humidity of Zone 1C was monitored over the same period.

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Microbiological analysis

All plates were incubated at 37°C for 48 h. Colonies were enumerated and sub-cultured before identification by matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonik MALDI biotyper; Bruker, Bremen, Germany). Those identified as *S. capitis* were tested for phenotypic susceptibility to cefoxitin, rifampicin, fusidic acid, ciprofloxacin and gentamicin via disk diffusion following EUCAST guidelines. Isolates with an antibiogram typical of the NRCS-A clone (i.e. resistance to cefoxitin, fusidic acid and gentamicin) were submitted to the UK National *Staphylococcus* and *Streptococcus* Reference Unit and were confirmed as being NRCS-A via whole genome sequencing.

Ethics statement

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Ethical approval was not required as these data were used by the organisations involved for a public health purpose of conducting communicable disease outbreak investigations. No patient specimens or data were collected. Written approval to carry out environmental sampling was given by each hospital site.

Laboratory Studies

Survival on plastic over time

Three NRCS-A and three non NRCS-A *S. capitis* isolates were cultured and maintained on Tryptone Soya Agar (TSA; E & O Laboratories Ltd). Broth cultures were prepared by transferring a single bacterial colony to 5 mL tryptic soy broth (TSB; Biomérieux UK Ltd, Hampshire, UK) and incubating overnight (18 h) at 37°C. Turbidity was adjusted to 0.36 (OD_{600}) using a low nutrient medium (LNM; tryptone (1 g/L) and sodium chloride (8.5 g/L)) before the bacterial suspension was diluted ten-fold. This resulted in a test suspension with a concentration of 10^7 colony forming units (cfu)/mL.

Plastic discs (diameter: 2cm; thickness: 0.2cm) were disinfected with 70% (v/v) isopropyl alcohol (IPA), rinsed with sterile water, and placed in a class II biological safety cabinet to dry. Following disinfection, discs were placed in empty, sterile petri dishes (5 discs per dish) and inoculated with 10µl of test suspension (~10^5 cfu). Immediately following inoculation (within 5 min), the petri dishes were transferred to an environmental (climatic) chamber (Caron Products and Services, Ohio, USA) programmed to simulate conditions within neonatal intensive care. The lids of the petri dishes were removed exposing *S. capitis* to an environment that replicated either a humidified neonatal incubator (36°C; 80% r.h) or an open bay (24°C; 40% r.h).

Immediately after inoculation (Time 0) or at pre-determined time points (1 day, 3 days and 7 days), five discs were removed from the environmental chamber. Each individual disc was aseptically transferred to a 30 mL plastic container containing 5 mL LNM and four sterile glass beads (3mm diameter). The disc was vortexed to remove bacteria from the disc surface and the resulting suspension serially diluted (ten-fold) using LNM. Aliquots (100 µL) of each dilution were cultured on TSA (37°C for 48 h) and the number of recovered colonies expressed as cfu/disc. Survival of *S. capitis* NRCS-A over time was compared to that of non-NRCS-A isolates.

Bactericidal activity of disinfectants

A quantitative suspension test (BS EN 1276:2019) was used to determine the efficacy of chemical disinfectants against NRCS-A *S. capitis*, non-NRCS-A *S. capitis* and the standard test organism, *S. aureus* NCTC 10788. Test products included sodium troclosene (1000 ppm available chlorine), a

chlorine dioxide-based surface disinfectant (produced by mixing citric acid (5%) with a sodium chlorite solution (2.1%) and diluting (50-fold) with water) and a QAC-based detergent/disinfectant (extracted from general-purpose "universal" wipes and containing 2-phenoxyethanol (≤0.6%), benzalkonium chloride (≤0.6%), didecyldimethylammonium chloride (≤0.6%) and biphenyl-2-ol, (≤0.1%)). All products were evaluated (in triplicate) under ambient conditions (22°C - 24°C) and in the presence of bovine albumin solution (3 g/L). Effectiveness was assessed after a contact time of 1- and 5 minutes. After neutralization (5 min), aliquots of the solution were cultured (in duplicate) on TSA (37°C for 48 h) and the number of colonies compared to that recovered from the test suspension prior to disinfection. Bactericidal activity was expressed as $\log_{10}$ reduction in viability.

Depending on the active agent, different products require different neutralization. Prior to performing the quantitative suspension test, appropriate neutralizing solutions were identified and validated [10]. Effective neutralization of sodium troclosene and the chlorine dioxide-based surface disinfectant was achieved using a solution comprising Tween 80 (30 g/L), sodium thiosulphate (3 g/L), lecithin (3 g/L) and catalase (0.25 g/L). Quadruple-strength Dey/Engley (D/E) neutralizing broth (Sigma-Aldrich Ltd, Gillingham, UK) was used to neutralize the QAC-based detergent/disinfectant. All neutralizing solutions were non-toxic to *S. capitis*.

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Analysis

Data analysis was performed using GraphPad Prism version 9.3.1 (2021). Statistical association between categorical variables was obtained by Fisher's exact tests. Quantitative data was analysed using t-tests.

Results

Field Study

Environmental sampling was carried out within four NICUs (A – D) between August 2021 and July 2022. One NICU was visited on two separate occasions (visits A(i) and A(ii)) and two NICUs were visited when a neonate known to be colonised with *S. capitis* NRCS-A was present on the unit (visits A(i) and B). Two NICUs had not reported clinical detection of the NRCS-A clone within the past 12-months (visits C and D). The mean temperature and relative humidity recorded within the four NICU environments was 24.1°C (range: 22.1°C to 26.9°C) and 46.4% (range: 41.6% to 62.7%), respectively.

S. capitis was recovered from 40 (21.1%) of 173 environmental surfaces. Of these, 30 (75%) harboured the NRCS-A clone, with surfaces located within zones 1A and 1B (i.e. either in or around the infant incubator) significantly more likely to be contaminated than surfaces located elsewhere within the

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NICU ($p = 0.001$; Table I). Incubators and other bedside equipment were contaminated with *S. capitis* NRCS-A regardless of known clinical detection(s). When sampling was carried out within the bedspace of a known colonised infant (visits A(i) and B), six (37.5%) of 16 surfaces sampled within zones 1A and 1B were contaminated with NRCS-A. When sampling focused on spaces occupied by non-colonised neonates admitted to the same two units, the NRCS-A clone was recovered from 7 (24.1%) of 29 surfaces sampled. Similarly, when sampling was carried out on units with no known colonisations or previous detections (visits A(ii), C and D), *S. capitis* NRCS-A was recovered from 15 (16%) of 94 surfaces; a recovery rate not significantly different to that observed when known NRCS-A cases were present at the time of sampling (19%; $p = 0.69$; Table I).

Four neonatal incubators were unoccupied at the time of sampling (visits A(i), A(ii), B and C). Of these, one was sampled both before and after terminal cleaning (visit A(i)) when, prior to cleaning, *S. capitis* NRCS-A was only recovered from two internal surfaces - the fan and humidifier port. The fan remained contaminated despite terminal cleaning and, whilst *S. capitis* was not recovered from the humidifier port following cleaning, NRCS-A was recovered from the exterior surface of the incubator implying ineffective cleaning and/or cross-contamination during the cleaning process.

S. capitis was recovered from 8 (27.6%) of the 29 settle plates placed in zones 1C, 2 and 3. Of these, one (collected during visit C) harboured isolates with an antibiogram typical of the NRCS-A clone (i.e. resistance to cefoxitin, fusidic acid and gentamicin).

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Laboratory Studies

Survival on plastic over time

The survival of *S. capitis* when incubated under environmental conditions designed to simulate the inside (36ºC; 80% r.h) and outside (24ºC; 40% r.h) of a humidified neonatal incubator was assessed over time. Survival of strains belonging to the NRCS-A clone (n=3) was compared to that of non-NRCS-A strains (n=3). Figure 2 illustrates the mean number of viable cells recovered from plastic discs up to 7 days after inoculation in a low nutrient medium (LNM).

S. capitis, regardless of strain or environmental condition, survived for 3 days with minimal losses in viability (0.23 to 0.49 $\log_{10}$ reductions). When incubated under conditions that replicated a NICU bay (24ºC; 40% r.h), cell numbers continued to decline slowly, with $\log_{10}$ reductions of 0.6 (NRCS-A) and 1.19 (non NRCS-A) over the 7-day study period (Figure 2). When exposed to the higher temperature and relative humidity, recovered cell numbers declined more rapidly and after 7-days, mean losses in viability of the NRCS-A and non NRCS-A strains equated to 2.1 and 3.52 $\log_{10}$ values respectively. After 7-days in LNM, the number of surviving NRCS-A cells was significantly greater than those of non NRCS-A strains regardless of environmental condition ($p < 0.02$).

Bactericidal activity of disinfectants

Sodium troclosene (1000 ppm available chlorine) and the QAC-based disinfectant/detergent (extracted from general purpose wipes) reduced *S. capitis* to below detectable levels (i.e. achieved a > 5 log reduction) within 1-minute. Effectiveness of the chlorine dioxide-based surface disinfectant increased with increasing contact time. However, whilst it reduced non NRCS-A *S. capitis* to undetectable levels within 5 minutes, it failed to eliminate either of the NRCS-A strains within the required (5 min) contact time (Table II).

Discussion

Over the last decade, the NRCS-A clone of *S. capitis* has become increasingly associated with late-onset-sepsis in low birthweight newborns. Understanding whether the healthcare environment can contribute to NRCS-A infections, incidents and outbreaks is key to developing and implementing effective control measures.

Zoning strategies can be used to identify environmental reservoirs and to focus control efforts on vulnerable areas [9]. In this study, environmental sampling was carried out in four NICUs. Each NICU was divided into zones of increasing distance from the neonate (Figure 1) and whilst *S. capitis* was recovered from surfaces across the neonatal setting (i.e. from within zones 1-3), the NRCS-A clone was rarely recovered from outside zone 1A (i.e. within the incubator) and zone 1B (i.e. the immediate neonatal bedspace).

As in previous studies [6-8], when samples were taken from within the immediate environment of a known positive case (visits A(i) and B), NRCS-A was frequently recovered from in and around the incubator, specifically the stethoscope, the porthole lock and the blanket covering the top of the incubator. Bacteria are readily transferred from a patient's skin to the stethoscope diaphragm during use [11] and the porthole lock and incubator cover are likely to be the first surfaces touched by care staff following patient contact. So, whilst these results are not unexpected, they do reinforce the need for frequent cleaning of patient monitoring equipment and incubators.

In this study, bedspaces adjacent to those of known NRCS-A cases were also contaminated. NRCS-A was recovered from surfaces within zone 1B (e.g. incubator porthole lock; monitor; computer keyboard) and from multi-user equipment (milk warmer) and furniture (equipment trolley) located within the same bay (zone 1C). It was hypothesised that NRCS-A had been transferred from the colonised neonate to surfaces elsewhere within the NICU via healthcare worker hands and whilst this highlights the importance of appropriate glove use and compliance with the five-moments for hand hygiene, the lack

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of epidemiological information and longitudinal sampling for NRCS-A isolates meant such transmission events could not be confirmed.

The presence of *S. capitis* NRCS-A on surfaces around non-infected neonates has been observed previously. Butin et al [7] sampled the environment of infected and non-infected patients in the same NICU and recovered NRCS-A from 57% and 33% of surfaces respectively. However, the colonisation status of the non-infected patients was not known, and the authors were unable to determine if the patients were the source of contamination or if the contaminated environment was a potential source of transmission. In the current study, three different NICUs were visited when there were no known colonisations. Two of these units (visit C and D) had not reported clinical detection of the NRCS-A clone within the past 12-months. However, NRCS-A was detected in all three NICUs and was recovered from 20% (14/68) of the surfaces sampled within zones 1A and 1B (Table I). Eight (57%) of these surfaces were associated with two incubators occupied by the same patient; one was occupied by the baby at the time of sampling, the other had been vacated after 7 days of use and was sampled prior to terminal cleaning and disinfection (visit A(ii)).

NRCS-A strains have been shown to commonly colonise uninfected babies [12] and, whilst the extent of localised contamination (stethoscope, mattress, inner and outer surfaces of both incubators) would suggest a recent and on-going source of contamination, the baby was not identified as being colonised or infected with *S. capitis* at any point during their admission. It is possible that the incubator(s) had previously housed a colonised patient and that residual surface contamination had not been removed by cleaning and disinfection processes.

When assessed under laboratory conditions and in comparison to the standard test organism, there was no evidence to suggest that the QAC-based detergent/disinfectant or sodium troclosene (two products used during the daily and/or terminal cleaning) would be ineffective against *S. capitis* (Table II). However, the quantitative suspension test used during this study has its limitations and results from laboratory studies may not translate to the clinical setting.

Disinfection failures have been reported, in part, due to incubator design. Two studies reporting the effectiveness of a QAC-based disinfection process recovered coagulase-negative staphylococci [8] and *S. capitis* NRCS-A [7] from 90% (18/20) and 63% (10/16) of incubators sampled both before and immediately following disinfection. In both cases, the inability to dismantle and immerse surfaces such as the mattress and scale was identified as a reason for persistent microbial contamination.

The presence of organic material can facilitate the survival of microorganisms and can inhibit the action of disinfectants. The use of pre-impregnated detergent and/or disinfecting wipes is a common method of cleaning high-touch surfaces in NICUs and elsewhere. The mechanical action applied when wiping helps to remove organic debris including microorganisms from a surface. However, if the solution released by the wipe lacks microbiocidal activity, cross-contamination during the cleaning process can

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occur. Whilst the quantity and concentration of active ingredient are important efficacy indicators (Table II) so too is the amount of solution that remains on the surface after wiping [13].

Despite its limitations, the quantitative suspension test can provide evidence to suggest that a disinfectant may be ineffective against a given target. In this study, the chlorine dioxide-based solution was less effective against the two NRCS-A strains than the non-NRCS-A isolate (Table II). Both NRCS-A isolates contained the *qacA* gene which is known to encode a multi-drug efflux pump. However, *qacA* is not statistically associated with NRCS-A [12] and other factors may have contributed to this increased tolerance.

If not removed from a surface and in the absence of effective disinfection, organisms can adsorb and adhere to a surface. In comparison to other *S. capitis* clades, the *tarIJL* genes are statistically associated with NRCS-A and are involved in the biosynthesis of teichoic acids [12], thought to be important in the initial attachment of gram-positive bacteria to biotic and abiotic surfaces [14, 15]. The ability of the NRCS-A clone to adhere to a surface and to persist under conditions of nutritional and desiccation stress has been demonstrated in this study (Figure 2) and by others [16]. Bacterial stresses may enhance the production of extracellular polysaccharides and biofilms containing staphylococci have been detected on surfaces within the healthcare setting [17] and have been shown to be more resistant to biocides including sodium hypochlorite [18].

Conclusion

Environmental sampling was carried out in four NICUs. Whilst it is acknowledged that sampling frequency was low and that the results represent a snapshot in time and place, the findings support those of previous studies and confirm that NRCS-A *S. capitis* can be readily recovered from the NICU environment with an increased likelihood of detection in areas that have direct exposure to infected or colonised infants (Table I). Commonly used surface disinfectants are likely to be effective against *S. capitis* (Table II), but these must be applied and used appropriately. Sub-optimal cleaning and/or difficult-to-clean surfaces could lead to persistence of *S. capitis* NRCS-A and whilst the role of dry surface biofilms in transmission has yet to be established, laboratory studies have demonstrated that these surface-associated bacteria can be transferred via hands to other fomites [19].

A large case-control study implemented as part of the incident response has not found an increase in mortality in infants with invasive *S. capitis* infection compared to those with other coagulase-negative staphylococcal infections (manuscript in peer review). Regardless, the widespread detection of the NRCS-A clone has highlighted a need for national guidance on cleaning within the NICU setting which should be delivered in conjunction with good IPC practice. An improvement in the routine disinfection of incubators is required [8] and active engagement with manufacturers of incubators to improve surface

design should be considered. Standardised cleaning protocols are needed not just for incubators and areas located close to the baby [20] but also for equipment used within the wider care area (Table I; [7]). Highly vulnerable patients with additional risk factors (e.g. invasive procedures and devices, immunological immaturity, antibiotic exposure) means NICUs are high-risk environments. Evidence-based recommendations to prevent fomite transmission (regardless of pathogen) will help ensure a safe environment for neonates during this vulnerable period.

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Conflicts of interest

The authors do not have any conflicts of interest

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Table I: Number (%) and zonal distribution of environmental surfaces harbouring *S. capitis* NRCS-A within four neonatal intensive care units with and without known *S. capitis* colonised/infected neonates.

	Number of positive surfaces / total number of surfaces sampled (%)		
	Known NRCS-A colonised neonate on unit during visit (A(i) and B)	No known NRCS-A colonised neonates on unit during visit (A(ii), C and D)	All visits (A - D)
Zone 1A	4/16 (25.0)	6/20 (30.0)	10/36 (27.8)
Zone 1B	9/29 (31.0)	8/48 (16.7)	17/77 (22.1)
Zone 1C	2/16 (12.5)	0/20 (0.0)	2/36 (5.6)
Zones 2 and 3	0/18 (0.0)	1/6 (16.7)	1/24 (4.2)
All zones	**15/79 (19.0)**	**15/94 (16.0)**	**30/173 (17.3)**

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Table II. Bactericidal activity (log_{10} reduction in viability) of commonly used surface disinfectants against *S. capitis* and the standard test organism *S. aureus* NCTC 10788. All products were evaluated (in triplicate) under ambient conditions (22ºC - 24ºC) and in the presence of bovine albumin solution (3 g/L). Efficacy was assessed after a contact time of 1- and 5 min.

chemical disinfectant	contact time	**log_{10} reduction in viability** *S. capitis* non NRCS-A clone	*S. capitis* NRCS-A clone	*S. capitis* NRCS-A clone	*S. aureus* NCTC 10788
QAC-based detergent/disinfectant [a]	1 min	> 5.10	> 5.11	> 5.49	> 5.31
sodium troclosene (1000 ppm available chlorine)	1 min	> 5.20	> 5.06	> 5.06	> 5.34
chlorine dioxide-based surface disinfectant [b]	5 min	> 5.25	< 3.66	< 3.73	4.3

[a] extracted from general-purpose "universal" wipes (2-phenoxyethanol (≤0.6%), benzalkonium chloride (≤0.6%), didecyldimethylammonium chloride (≤0.6%) and biphenyl-2-ol, (≤0.1%)).

[b] produced by mixing 50 mL citric acid (5%) with 50 mL sodium chlorite solution (2.1%) and diluting (50-fold) with water

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Figure 1: Environmental sampling was carried out within zones of increasing distance from the infant. Zone 1A: internal surfaces of incubator. Zone 1B: immediate bedspace (including external surfaces of incubator). Zone 1C: wider NICU bay. Zone 2: areas used by families and staff. Zone 3: staff only areas

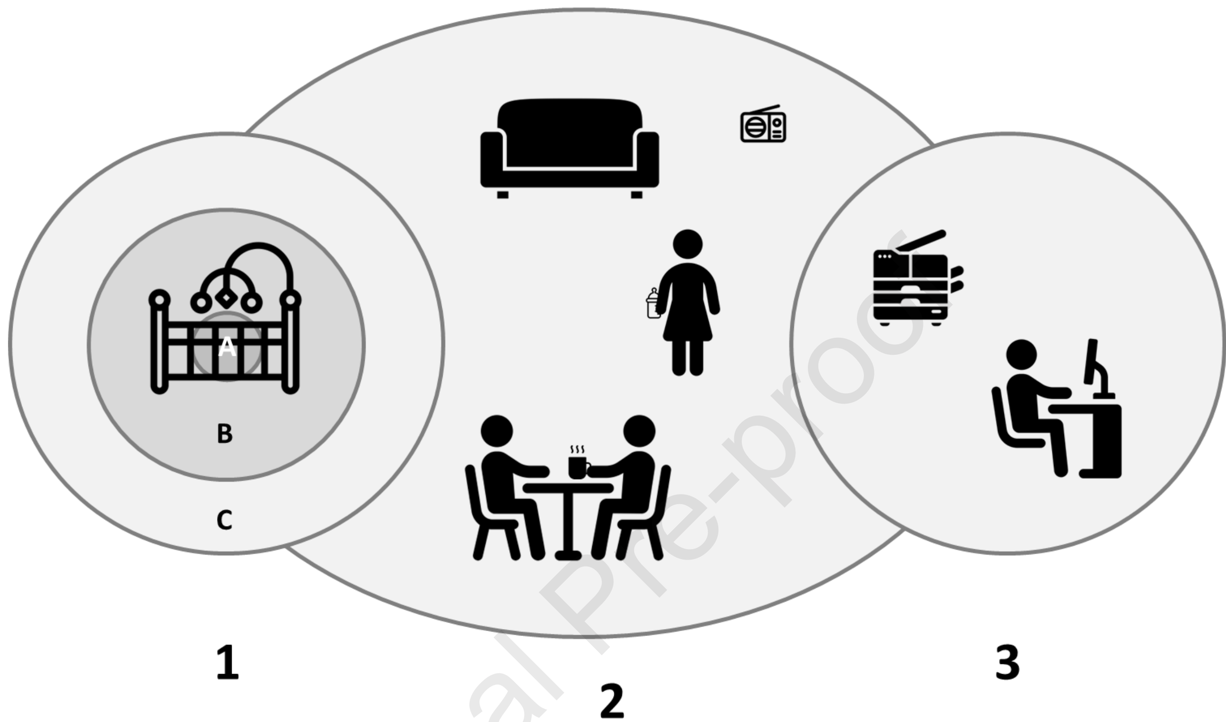

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Figure 2: Recovery of *Staphylococcus capitis* from plastic surfaces. NRCS-A isolates (n=3) and non-NRCS-A isolates (n=3) were suspended in a low nutrient medium and held for up to 7-days under environmental conditions designed to simulate the inside (36°C; 80% r.h) and outside (24°C; 40% r.h) of a humidified infant incubator. The dotted line represents the detection limit of the assay.