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Risk factors for *Staphylococcus capitis* pulsotype NRCS-A colonisation among premature neonates in the neonatal intensive care unit of a tertiary-care hospital: a retrospective case-control study

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SUMMARY

Background: A *S. capitis* strain called NRCS-A (*S. capitis* NRCS-A) has emerged as a cause of bloodstream infections and sepsis in neonatal intensive care units (NICUs) worldwide.
Aim: To identify risk factors for *S. capitis* NRCS-A colonisation among neonates, Dunedin Hospital NICU, Dunedin, New Zealand, from September 2013 through March 2015.
Methods: Weekly axillary swabs categorised eligible neonates as a case or a control. A case was defined as a week ending with a neonate's first positive swab for *S. capitis* NRCS-A and a control as a week in which a neonate remained negative. Weekly exposures were abstracted from hospital medical records. Analyses were performed using conditional logistic regression.
Findings: The median (range) gestational age at birth of participants was 32.7 (23.1—41.3) weeks. Participants contributed 26 weeks of case data and 177 weeks of control data. On adjusted analysis compared with matched controls, cases had higher odds of requiring invasive mechanical ventilation (OR 3.6, 95% CI: 1.1—11.6, p=0.035) and of a patent ductus arteriosus (PDA) (OR 3.0, 95% CI: 1.0—9.0, p=0.044). Cases had lower odds of being part of a multiple birth (OR 0.24, 95% CI 0.08—0.73, p=0.001), having an area of inflamed skin (OR 0.31, 95% CI: 0.13—0.75, p=0.009), and specifically an area of inflamed axillary skin (OR 0.08, 95% CI: 0.01—0.50, p=0.006).
Conclusions: We found that premature neonates with invasive mechanical ventilation and PDA had greater odds for *S. capitis* NRCS-A colonisation. Transmission may be mediated by increased staff contact, but prospective research is needed to confirm this.

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Introduction

In 2012, methicillin-resistant *S. capitis* pulsotype NRCS-A (*S. capitis* NRCS-A), a coagulase negative *Staphylococcus* (CoNS), was identified as a cause of bloodstream infections among neonates in a neonatal intensive care unit (NICU) in France, and was named after the National Reference Centre for Staphylococci at the University Hospital in Lyon, France [1]. Since identification, *S. capitis* NRCS-A has been isolated from NICU patients elsewhere in France and worldwide, but rarely from adults [1,2]. The homology between the strains isolated from geographically diverse NICUs suggested global dissemination of the *S. capitis* NRCS-A clone [2]. *S. capitis* NRCS-A is associated with up to 40% of bloodstream infections [1,3]. The strain is usually resistant to methicillin and aminoglycosides, has decreased susceptibility to vancomycin, and can form biofilms [4].

Risk factors for bloodstream infection and sepsis among neonates, such as the presence of intravascular catheters, are well established for CoNS [3,5]. However, there has been little research on risk factors for *S. capitis* NRCS-A colonisation. Since colonisation is often a prerequisite for infection, understanding reservoirs, sources, and modes of transmission of staphylococci in the NICU setting is crucial to the design of strategies to interrupt transmission.

Based on our understanding of the transmission of CoNS generally [6—10], we developed two broad hypotheses on the mode of transmission. We sought to investigate the role of direct contact or by maternal milk or formula, and selection of *S. capitis* NRCS-A by the use of antimicrobials.

Methods

Study setting

Dunedin Hospital, Dunedin, New Zealand, is a tertiary-care hospital serving a catchment population of approximately 315,000 residents. *S. capitis* NRCS-A was first isolated in the Dunedin Hospital NICU in 2007 from a neonate with a bloodstream infection [11] and has persisted in the NICU since, including following relocation of NICU services to a renovated facility within the same hospital in December 2014. The current Dunedin Hospital NICU has 13 rooms and 21 bed spaces with one, two, or four bed spaces per room. The standard initial antimicrobial regimen for suspected congenital sepsis in the unit is amoxicillin and gentamicin. The standard initial antimicrobial regimen for late neonatal sepsis is amoxicillin and amikacin. The endemic *S. capitis* NRCS-A is resistant to beta-lactams, gentamicin, and fusidic acid but remains susceptible to amikacin and vancomycin. Ethics approval was obtained from the University of Otago Human Ethics Committee (Health) (reference number HD16/050).

Study design

We performed a retrospective nested case-control study among neonates admitted to the Dunedin Hospital NICU. Abstraction of retrospective data took place from 12 December 2017 through 16 February 2018. A nested case-control study design was chosen in order to investigate the effect of recent exposures. A surveillance program seeking to identify NICU patients with *S. capitis* colonisation was established by the Dunedin Hospital Infection Prevention and Control Service in September 2013 following a cluster of bloodstream infections caused by *S. capitis*. An axillary swab (COPAN Transystem™, COPAN Italia, Brescia, Italy) was sought every Monday throughout the neonate's admission in the NICU. Isolation and identification of *S. capitis* was performed by Southern Community Laboratories, Dunedin, New Zealand. Swabs were plated on trypticase soy agar with 5% sheep blood (Fort Richard Laboratories, Auckland, New Zealand) and incubated in ambient air at 37°C overnight. Several colonial variants suspicious for *S. capitis* were picked and identified by matrix associated laser desorption ionisation time of flight mass spectrometry (MALDI-ToF-MS) (Bruker Daltonics, Massachusetts, USA). Cultures were confirmed as the NICU-specific *S. capitis* NRCS-A strain by pulse-field gel electrophoresis at the Institute of Environmental Science and Research, Wellington, New Zealand. All neonates who were admitted to the Dunedin Hospital NICU from 9 September 2013 through 9 March 2015, and who had consecutively received two or more weekly axillary swabs (8—14 days following admission), were eligible for inclusion in this study. Neonates were excluded from the study if their first axillary swab was positive for *S. capitis* NRCS-A, they were admitted to the NICU before the surveillance began, or they did not receive their scheduled second swab.

Data collection

We sought validated and widely used questionnaires from past studies of *Staphylococcus* spp. transmission and disease in NICU settings, and identified relevant questionnaires from the research team at the Prevention and Response Branch, Division of Healthcare Quality Promotion, US Centers for Disease Control and Prevention. The questionnaires were adapted to create case report forms to address potential risk factors for colonisation, sources, and modes of transmission (Appendix). The case report forms collected baseline sociodemographic and clinical data, as well as weekly exposures data including comorbidities, procedures, devices, antimicrobial and other medication use, number and type of enteral feedings, bed space, infant weight, number of cares by nurse or parents, equipment used during cares, and procedures performed by allied health professionals such as imaging procedures. Clinical data were sought from the neonate's hard copy and electronic medical and laboratory records.

For each eligible neonate, exposure data were collected from the neonate's first negative swab until their first positive swab result, they were discharged from the NICU, or they missed a swab collection. Axillary swabs were taken every Monday at 6am and exposures were investigated during the preceding one week period prior to swab collection. Axillary swab results on both the Monday of, and the Monday following

the exposure week were necessary to classify the neonate's exposure week as a case or control. A case was defined as a week in which an eligible neonate had a negative swab result on the Monday of the exposure week, and a positive swab result on the following Monday. A control was defined as a week in which an eligible neonate had a negative swab result on the Monday of the exposure week and all previous weeks, and a negative swab result on the following Monday. Eligible neonates could contribute multiple weeks of control data. However, eligible neonates could contribute case data only once, after which we stopped collecting data for the neonate.

Statistical analysis

Data were abstracte from the sources and entered into a REDCap (service version 8.2.0, Vanderbilt University, Tennessee, USA) online database hosted at the University of Otago [12]. Controls were matched to cases by exposure week on calendar time using risk-set sampling [13]. For each case, we matched all available controls for the four weeks prior to the case's first positive swab. An individual neonate could provide multiple weeks of control data per case, for as many cases as applicable. Individual neonates could provide both case and control week data but an individual neonate could not serve as their own control. From individual neonates who contributed case or control data, we compared numbers and proportions of antimicrobial use among colonised and non-colonised neonates. A composite variable of antimicrobial exposure was created for antibacterials against which *S. capitis* was resistant: amoxicillin, cefotaxime, gentamicin, fusidic acid, and metronidazole.

For unadjusted and adjusted analyses, we used conditional logistic regression with robust standard errors to estimate matched odds ratios, 95% confidence intervals, and p-values. Unadjusted conditional logistic regression was performed for all variables which had three or more observations in each exposure category and had data for both cases and controls. The small sample size precluded a full multivariable analysis, so the analysis was adjusted for two major confounders in addition to calendar week: low gestational age (GA) at birth and length of stay. GA was categorised into $\leq$32 weeks and $>$32 weeks as $\leq$32 weeks' gestation defines very preterm delivery [14]. Length of stay was categorised into $\leq$40 days and $>$40 days. Length of stay varied with birth gestation, with most neonates discharged before their due date. The binary variables were chosen as data were limited and a linear relationship with the outcome on the logit scale was implausible. Adjusted conditional logistic regression was performed for all variables which had eight or more observations in each exposure category and had data for both cases and controls. All statistical analyses were performed using Stata/IC version 15.1 (StatCorp, College Station, TX, USA) [15].

Results

Of 352 neonates admitted to the NICU during the study period, 236 (66.3%) were swabbed at least once during their stay. Of these, 117 (49.6%) were eligible for participation. Reasons for not being swabbed included missing a scheduled swab or a NICU admission period not including a Monday. Of 119 (50.4%) ineligible neonates, 88 (73.3%) were swabbed once, 16 (13.3%) had a positive first swab, 9 (7.5%) were admitted to the NICU before the study began, and 6 (5.0%) did not receive their scheduled second swab (Fig. 1).

Of 117 eligible neonates, medical records were available for 64 (54.7%). Of 64 neonates with medical records, 26 (40.6%) were of neonates colonised with *S. capitis* NRCS-A and 38 (59.4%) were of neonates not colonised with *S. capitis*. NRCS-A Of 53 (45.3%) neonates without available medical records, 16 (30.2%) were of neonates colonised with *S. capitis* and NRCS-A 37 (69.8%) were of neonates not colonised with *S. capitis* NRCS-A. Of unavailable medical records, all were inaccessible due to asbestos contamination of medical record storage rooms in Dunedin Hospital [16].

Of 64 eligible neonates with available medical records, the mean (standard deviation) GA at birth was 32.7 (0.5) weeks, mean birthweight was 1,906 (108) g, and the mean length of stay was 37.4 (3.2) days. The 26 colonised neonates contributed one week of case data each. Following matching, 177 weeks of control data were matched to the 26 weeks of case data. The median (range) number of controls per case was 6.8 (1, 26). Among the 50 neonates contributing control data, 26 (52.0%) were never colonised neonates, and 24 (48.0%) were neonates who subsequently became cases. There were 14 (36.8%) never colonised neonates who did not contribute weekly data, through matching criteria. Of 177 weeks of control data, 94 (53.1%) weeks were contributed by neonates that became colonised, and 83 (46.8%) weeks were contributed by never colonised neonates. Of the 26 cases, 11 (42.3%) became positive for *S. capitis* NRCS-A during their first full week in the NICU, 6 (23.1%) during their second week, 4 (15.4%) during their third week, 4 (15.4%) during their fourth week, and 1 (3.8%) during their fifth week. No cases became positive for *S. capitis* NRCS-A after their fifth week in the NICU.

On unadjusted analysis, compared with controls, cases had greater odds of being born weighing $\leq$1500g (OR 4.1, 95% CI 1.1−15.7, p=0.040) and of being born at $\leq$32 weeks' GA (OR 3.6, 95% CI 1.2−10.6, p=0.019). Cases tended to have had a longer stay in NICU than controls (OR 1.7, 95% CI 0.66−4.3, p=0.271) (Table I), although this difference was not statistically significant.

On adjusted analysis, compared with controls, cases had reduced odds of being born as a part of a multiple birth (OR 0.14, 95% CI 0.04−0.44, p=0.001). Of the 26 cases, there were four pairs of twins. There was no statistically significant association with use of individual antimicrobial agents or with the composite variable of antimicrobial exposure with *S. capitis* NRCS-A colonisation (Table II). No cases used fusidic acid compared to 31 (17.5%) of the controls (OR undefined). No non-antimicrobial medications were associated with *S. capitis* NRCS-A colonisation on adjusted analysis. Comparing individual neonates that contributed case or control data, 23 (88.5%) of 26 colonised neonates used antimicrobials during their NICU admission compared with 7 (29.2%) of 24 non-colonised neonates (p$<$0.0005). Among twins, each neonate pair became colonised within one week of each other. In all cases when one twin become colonised the other also became colonised with *S. capitis* NRCS-A.

In terms of procedures and devices, compared with controls cases had increased odds of having had invasive mechanical ventilation (OR 3.6, 95% CI 1.1−11.6, p=0.035) (Table III). On adjusted analysis of medical history, compared with controls, cases had increased odds of having a patent ductus arteriosus

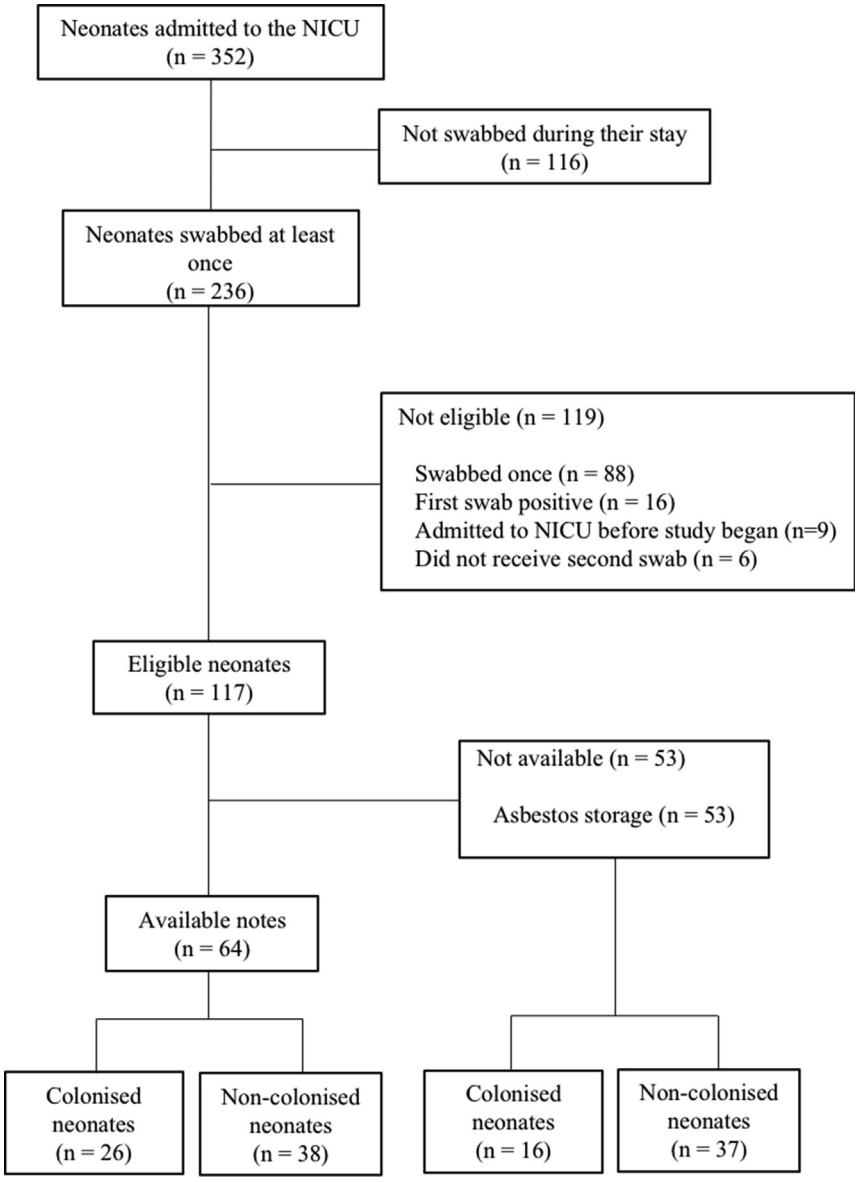

Figure 1. Flow diagram of the neonates and their eligibility for inclusion in the retrospective case-control study, Dunedin Hospital NICU, September 2013 through March 2015.

(PDA) (OR 3.0, 95% CI 1.0—9.0, p=0.044) (Table IV). Compared with controls, cases had lower odds of having an area of inflamed skin (OR 0.31, 95% CI 0.13—0.75, p=0.009), and of having an area of inflamed axillary skin (OR 0.08, 95% CI 0.01—0.50, p=0.006). Compared with controls, cases had lower odds of having enteral feeds with formula, although this was of borderline statistical significance (OR 0.29, 95% CI 0.08—0.99, p=0.05). There were no statistically significant associations of *S. capitis* colonisation with the neonate's weight, procedures performed outside the NICU or by allied health professionals, route of feeds, type of bed, bed space, or room.

Discussion

We demonstrate that in the Dunedin Hospital NICU, *S. capitis* NRCS-A colonisation was associated with the requirement for invasive mechanical ventilation and having a PDA. PDA has been associated with decreased blood flow velocity in the gut and feed intolerance [17,18]. Neonates with feed intolerance take longer to achieve full enteral feeds, which may affect the neonate's intestinal microflora and create a niche for *S. capitis* NRCS-A colonisation [17]. However, feed intolerance was not significantly associated with colonisation in this study (Table III). While it is possible that neonates could be colonised through contaminated invasive mechanical ventilation equipment, both neonates with requirement for invasive mechanical ventilation and those with PDA represent members of a group of more medically dependent and frequently handled neonates. The retrospective nature of data collection for this study meant that we were unable to measure the level of handling of neonates by healthcare workers or their exposure to medical equipment and instruments. However, we propose that the more frequent and prolonged contact required for both routine and

Table I
Baseline characteristics of cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n= 26		Matched controls n= 177		Unadjusted		p-value	Adjusted for GA and length of stay		p-value
	n	(%)	n	(%)	OR	(95% CI)		OR	(95% CI)	
Female	5	(19.2)	52	(23.4)	0.6	(0.19—1.0)	0.421	0.43	(0.11—1.6)	0.204
Ethnicity [a]										
Non-Māori	20	(76.9)	137	(77.4)	1.2	(0.37—4.0)	0.747	1.6	(0.4—6.4)	0.506
Māori	6	(23.1)	39	(22.0)	1	(reference)		1	(reference)	
Gestational age (GA)										
≤32 weeks	18	(69.2)	83	(46.9)	3.6	(1.2—10.6)	0.019	NA		
>32 weeks	8	(30.8)	94	(53.1)	1	(reference)				
Birth weight [b]										
≤1500g	19	(73.1)	88	(49.7)	4.1	(1.1—15.7)	0.040	1.8	(0.27—12.1)	0.548
>1500g	7	(26.9)	89	(50.3)	1	(reference)				
Birth										
Multiple births	8	(30.8)	105	(59.3)	0.24	(0.08—0.73)	0.012	0.14	(0.04—0.44)	0.001
Born in Dunedin Hospital	25	(96.2)	163	(92.1)	3.3	(0.34—32.1)	0.303	3.2	(0.36—27.6)	0.297
Delivery										
Caesarian section	20	(76.9)	140	(79.1)	2.2	(0.39—12.5)	0.376	1.1	(0.17—7.7)	0.891
Vaginal delivery with tools	0	(0.00)	7	(3.5)						
Vaginal delivery without tools	6	(23.1)	30	(17.0)	1	(reference)				
Length of stay										
≤40 days	11	(42.3)	84	(47.5)	1	(reference)				
>40 days	15	(57.7)	93	(52.5)	1.7	(0.66—4.3)	0.271	NA		
Vital status at discharge										
Deceased	1	(3.9)	0	(0.0)						

NICU — neonatal intensive care unit; GA — gestational age; NA — adjusted for gestational age and length of stay so no output included. Unadjusted and adjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=177) are all the controls from the matched case-control sets. Unadjusted analysis was not performed for observations with no cases, or cell numbers less than 3; Adjusted analysis was not performed for observations with no cases or cell numbers less than 8.

[a] Ethnicity missing for one control neonate.

[b] Birthweight was per 100g for the univariate analysis.

Table II
Unadjusted and adjusted analysis of antimicrobial use in cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

Antimicrobials	Cases n= 26		Matched controls n= 177		Unadjusted		p-value	Adjusted for GA and length of stay		p-value
	n	(%)	n	(%)	OR	95% CI		OR	95% CI	
Any antimicrobial	19	(73.1)	96	(54.2)	2.3	(0.57—8.9)	0.245	1.2	(0.20—7.1)	0.880
Antibacterials										
Amoxicillin	13	(50.0)	57	(32.2)	1.8	(0.60—5.4)	0.290	1.6	(0.49—5.3)	0.440
Gentamicin	8	(30.8)	30	(17.0)	1.6	(0.53—4.8)	0.403	1.8	(0.64—5.2)	0.262
Fusidic acid	0	(0.0)	31	(17.5)						
Metronidazole	1	(3.9)	27	(15.3)	0.21	(0.02—1.9)	0.165	0.07	(0.003—1.3)	0.073
Amikacin	4	(15.4)	19	(10.7)	1.4	(0.37—5.5)	0.600	0.74	(0.11—4.9)	0.758
Chloramphenicol	1	(3.9)	8	(4.5)	0.93	(0.11—8.1)	0.951	1.7	(0.15—18.0)	0.675
Cefotaxime	2	(7.7)	3	(1.7)	5.0	(0.38—65.3)	0.224	8.1	(0.47—140.3)	0.149
Augmentin	0	(0.0)	0	(0.0)						
Ceftazadime	0	(0.0)	0	(0.0)						
Erythromycin	0	(0.0)	0	(0.0)						
Penicillin	0	(0.0)	0	(0.0						
Vancomycin	0	(0.0)	0	(0.0)						
Antifungals										
Fluconazole	14	(53.9)	57	(32.2)	2.5	(0.87—7.0)	0.091	0.37	(0.03—4.5)	0.437
Nystatin	0	(0.0)	6	(3.0)						

(*continued on next page*)

Table II (*continued*)

Antimicrobials	Cases n= 26		Matched controls n= 177		Unadjusted		p-value	Adjusted for GA and length of stay		p-value
	n	(%)	n	(%)	OR	95% CI		OR	95% CI	
Clotrimazole	0	(0.0)	2	(1.0)						
***S. capitis* resistance**										
Composite [a]	13	(50.0)	70	(39.6)	1.4	(0.45—4.3)	0.566	1.1	(0.45—3.9)	0.864

NICU — neonatal intensive care unit; GA — gestational age.
Unadjusted and adjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=177) are all the controls from the matched case-control sets. Unadjusted analysis was not performed for observations with no cases, or cell numbers less than 3; Adjusted analysis was not performed for observations with no cases or cell numbers less than 8.
[a] Composite antimicrobial includes antibacterials against which *S. capitis* has resistance: amoxicillin, cefotaxime, gentamicin, fusidic acid, and metronidazole.

Table III
Unadjusted and adjusted analysis of the procedures and devices required by cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n= 26		Matched controls n= 177		Unadjusted		p-value	Adjusted for GA and length of stay		p-value
	n	(%)	n	(%)	OR	95% CI		OR	95% CI	
Nasogastric tube [a]	16	(61.5)	127	(71.8)	0.49	(0.13—1.9)	0.301	1.2	(0.17—8.2)	0.866
Peripheral IV cannula	16	(61.5)	108	(61.0)	0.57	(0.15—2.1)	0.399	0.42	(0.09—1.9)	0.267
Orogastric tube [b]	16	(61.5)	94	(53.1)	1.4	(0.36—5.5)	0.619	0.28	(0.05—1.47)	0.133
Nasal CPAP	14	(53.9)	98	(55.4)	1.1	(0.36—3.6)	0.828	0.26	(0.06—1.2)	0.089
PICC	16	(61.5)	62	(35.0)	2.7	(0.72—9.9)	0.143	0.65	(0.04—10.2)	0.760
UVC	7	(26.9)	17	(9.6)	2.3	(0.68—7.9)	0.177	1.5	(0.54—4.1)	0.440
UAC	3	(11.5)	6	(3.4)	2.9	(0.55—14.8)	0.211	1.5	(0.21—10.2)	0.694
Invasive mechanical ventilation	5	(19.2)	4	(2.3)	5.8	(1.7—19.7)	0.005	3.6	(1.1—11.6)	0.035
Endotracheal intubation	2	(7.7)	3	(1.7)	3.9	(1.0—15.2)	0.049			
Nasal cannula	0	(0.0)	5	(2.8)						
ROP screen	0	(0.0)	4	(2.3)						
Blood tests										
Number, mean (sd)	12.2	(±2.3)	8.0	(±0.6)	1.0	(1.0—1.1)	0.074	0.99	(0.94—1.0)	0.744
Phototherapy										
None	16	(61.5)	119	(67.2)	1	(reference)		1	(reference)	
1—2 days	5	(19.2)	50	(28.3)	0.48	(0.17—1.3)	0.158	0.52	(0.19—1.4)	0.282
3—6 days	5	(19.2)	8	(4.5)	3.5	(1.1—11.0)	0.033	2.5	(0.71—8.4)	0.129
RBC transfusion										
Number, mean (sd)	1.2	(±0.2)	1.5	(±0.3)						
Apnoeas [c]										
Number, mean (sd)	8.8	(±1.8)	0.56	(±0.04)	1.4	(0.60—3.3)	0.430	0.70	(0.28—1.8)	0.455
Type of stimulation										
Gentle	15	(57.7)	93	(52.5)	1.6	(0.64—3.8)	0.334	0.80	(0.30—2.1)	0.647
Moderate	3	(11.5)	35	(19.8)	0.55	(0.14—2.2)	0.401	0.24	(0.04—1.3)	0.104
Vigorous	0	(0.0)	7	(4.0)						
Funnel/facial O_2	11	(42.3)	87	(49.2)	1.0	(0.43—2.5)	0.938	0.43	(0.15—1.3)	0.122

NICU — neonatal intensive care unit; GA — gestational age; IV — intravenous; CPAP — continuous positive airway pressure; PICC — peripherally inserted central catheter; UVC — umbilical vein catheter; UAC — umbilical artery catheter; ROP - retinopathy of prematurity; sd — standard deviation; O_2 — oxygen.
Unadjusted and adjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=177) are all the controls from the matched case-control sets. Unadjusted analysis was not performed for observations with no cases, or cell numbers less than 3; Adjusted analysis was not performed for observations with no cases or cell numbers less than 8.
[a] One case missing data.
[b] One control missing data.
[c] All apnoeas required stimulation, some neonates required more than one type of stimulation per apnoea.

Table IV
Unadjusted and adjusted analysis of the medical history of cases and controls, Dunedin Hospital NICU, September 2013 through March 2015

	Cases n= 26		Matched controls n= 177		Unadjusted		p-value	Adjusted for GA and length of stay		p-value
	n	(%)	n	(%)	OR	95% CI		OR	95% CI	
Feed intolerance	6	(23.1)	50	(28.3)	0.48	(0.14–1.8)	0.272	0.27	(0.07–1.1)	0.064
Gastric aspirate	6	(23.1)	49	(27.7)	0.59	(0.16–2.2)	0.429	0.37	(0.09–1.6)	0.180
Temperature instability [a]	13	(50.0)	42	(23.7)	2.8	(1.2–6.7)	0.022	3.8	(0.80–18.4)	0.094
Skin injury	5	(19.2)	34	(19.2)	0.98	(0.34–2.8)	0.973	0.69	(0.24–2.0)	0.495
Sepsis workup	5	(19.2)	23	(13.0)	1.8	(0.68–4.9)	0.234	1.7	(0.65–4.2)	0.291
Intracranial haemorrhage	0	(0.0)	11	(6.2)						
Hypoglycaemia	0	(0.0)	10	(5.7)						
Renal impairment	0	(0.0)	10	(5.7)						
Flaky skin	1	(3.9)	6	(3.4)	0.65	(0.04–10.1)	0.761			
Sepsis diagnosis	2	(7.7)	2	(1.1)	10.4	(0.81–134.2)	0.072			
Neonatal encephalopathy	0	(0.0)	4	(2.3)						
Umbilical flare	1	(3.9)	2	(1.1)						
Retinopathy of prematurity	0	(0.0)	0	(0.0)						
Area of inflamed skin										
Yes, total	8	(30.8)	105	(59.3)	0.28	(0.11–0.73)	0.009	0.31	(0.13–0.75)	0.009
Axilla	2	(7.7)	41	(23.2)	0.28	(0.05–1.5)	0.132	0.08	(0.01–0.50)	0.006
Eye	3	(11.5)	27	(15.3)	1.0	(0.24–4.5)	0.954	2.3	(0.34–15.1)	0.392
Buttocks	2	(7.7)	26	(14.7)	0.34	(0.07–1.7)	0.195	0.85	(0.13–5.6)	0.863
Intravenous line	1	(3.9)	20	(11.3)	0.31	(0.04–2.6)	0.278	0.25	(0.05–1.4)	0.116
Ear	0	(0.0)	6	(3.4)						
Full body	0	(0.0)	4	(2.3)						
Groin	1	(3.9)	3	(1.7)	1.7	(0.26–11.7)	0.570			
Oral thrush	0	(0.0)	3	(1.7)						
Neck	1	(3.9)	2	(1.1)						
Enteral feedings										
Expressed breast milk	23	(88.5)	165	(81.3)	1.7	(0.50–5.6)	0.4	3.27	(0.68–15.82)	0.1
Breast milk	6	(23.1)	48	(23.7)	1.1	(0.3–3.2)	0.9	2.64	(0.70–9.92)	0.2
Donor breast milk	0	(0.0)	0	(0.0)	NA					
Human milk fortifier	6	(23.1)	55	(27.1)	1.6	(0.50–5.3)	0.4	1.44	(0.36–5.83)	0.6
Formula	6	(23.1)	100	(49.3)	0.34	(0.13–0.88)	0.03	0.29	(0.08–0.99)	0.05
Gastrointestinal disease										
Yes, total	0	(0.0)	22	(12.4)						
Necrotising enterocolitis	0	(0.0)	12	(6.8)						
Bowel ischaemia	0	(0.0)	4	(2.3)						
Cardiac abnormalities										
Yes, total	13	(50.0)	46	(26.0)	2.9	(1.2–7.3)	0.020	2.4	(0.90–6.5)	0.079
Patent ductus arteriosus	12	(46.2)	24	(13.6)	4.4	(2.0–10.0)	<0.001	3.0	(1.0–9.0)	0.044
Murmur	5	(19.2)	31	(17.5)	1.0	(0.37–2.7)	1.000	0.83	(0.26–2.6)	0.748
Septal defects	1	(3.9)	12	(6.8)	0.78	(0.08–7.5)	0.829	0.57	(0.05–6.9)	0.661
Ventricular impairment	1	(3.9)	7	(4.0)	1.1	(0.11–10.9)	0.943	3.2	(0.28–36.1)	0.356
Artery impairment	0	(0.0)	2	(1.1)						
Valve impairment	1	(3.9)	0	(0.0)						
Tachycardia	1	(3.9)	0	(0.0)						
Pulmonary disease										
Yes, total	12	(46.2)	81	(45.8)	2.0	(0.51–7.6)	0.323	1.1	(0.35–3.5)	0.862
Respiratory distress syndrome	12	(46.2)	73	(41.2)	4.0	(0.80–19.8)	0.092	2.0	(0.45–9.2)	0.356
Chronic lung disease	4	(15.4)	8	(4.5)	7.4	(1.5–37.9)	0.016	3.5	(0.73–16.9)	0.118
Pulmonary hypoplasia	1	(3.9)	3	(1.7)	4.9	(0.75–32.5)	0.097			
Emphysema	1	(3.9)	0	(0.0)						

(*continued on next page*)

Table IV (*continued*)

	Cases n= 26		Matched controls n= 177		Unadjusted		p-value	Adjusted for GA and length of stay		p-value
	n	(%)	n	(%)	OR	95% CI		OR	95% CI	
Pneumothorax	1	(3.9)	0	(0.0)						
Pulmonary haemorrhage	0	(0.0)	1	(0.6)						

NICU — neonatal intensive care unit; GA — gestational age.
Unadjusted and adjusted OR, 95% CI and p-values were estimated using conditional logistic regression to account for the matching. Controls (n=177) are all the controls from the matched case-control sets. Unadjusted analysis was not performed for observations with no cases, or cell numbers less than 3; Adjusted analysis was not performed for observations with no cases or cell numbers less than 8.
[a] Temperature instability is defined by a neonate's requirement for external temperature control using incubator controls, or an adjustment of their amount of clothing.

emergency care of this group places them at greater risk for both healthcare worker and fomite transmission.

Neonates born as part of a multiple birth share microflora more often than non-multiple birth neonates, possibly due to shared maternal contact, including shared expressed breast milk [19,20]. We identified eight cases, four sets of twins, who were part of a multiple birth. Following colonisation of one twin, the co-twin became colonised within a week. This suggests that transmission of *S. capitis* NRCS-A between twins is common, despite our finding that being part of a multiple birth was associated with reduced odds of colonisation. Nursing care of twins is often cohorted, with one nurse looking after twins. In non-colonised twins this may decrease exposure to colonisation compared with non-twin infants, and where one twin is colonised it could contribute to the likelihood of transmission to the second twin. Alternatively, this may be an artefact of the analysis — for example, the matching on calendar time and the repeated use of control baseline data.

Both having an area of inflamed skin and an area of inflamed axillary skin was associated with lower odds of *S. capitis* NRCS-A colonisation. To our knowledge, no epidemiologic studies have investigated skin inflammation as a risk factor for *S. capitis* NRCS-A or CoNS colonisation of neonates. While evidence is lacking, it is possible that having inflamed skin, whether due to an infectious or non-infectious process, is hostile to *S. capitis* NRCS-A. However, this needs exploration in future research. Alternatively, the treatment of inflamed skin with topical antimicrobials, such as with fusidic acid, may prevent the growth of *S. capitis* NRCS-A, although we note the New Zealand NICU strain has previously been shown to be phenotypically resistant to fusidic acid due to the presence of the *fusB* gene on plasmid pSC16875 [11].

Studies have found that altered microflora was a risk factor for late onset neonatal sepsis (LONS), [21] including LONS due to vancomycin-resistant *S. capitis* [22]. Therefore, we hypothesised that antimicrobial exposure might be associated with *S. capitis* NRCS-A colonisation due to its impact on the skin and gut microflora [21,23]. When looking at the differences between individual neonates, our study showed that antimicrobial use was more common among colonised than non-colonised neonates. However, antimicrobial use was not statistically associated with *S. capitis* NRCS-A colonisation in our study. It is possible that antimicrobials were a risk factor for *S. capitis* NRCS-A colonisation but were not detected because the exposure period was too short to detect an effect.

Although borderline statistically significant, we found that enteral feeds with formula were associated with a reduced risk of *S. capitis* NRCS-A colonisation. Breast feeding represents a period of extended direct skin contact with the mother that does not occur during formula feeding. It is plausible that neonates fed with formula are protected against colonisation from bacteria in expressed breast milk, or from the maternal breast and skin. Against that, breast milk feeding was not associated with increased odds of colonisation in the adjusted analysis.

Our study has a number of limitations. The retrospective collection of exposure data meant that detailed information on exposure to healthcare workers, medical equipment and instruments, bed spaces, and movement around the NICU could not be collected. Furthermore, some data may have been inconsistently recorded, and potential confounders overlooked. Because of limited data on transmission of *S. capitis* NRCS-A in the NICU setting, we gathered data on a wide range of potential sources and modes of transmission, increasing the risk for type I error. The selection of a one week exposure period to investigate risk factors for colonisation was arbitrary and a longer exposure period may have been more appropriate but would have reduced the number of available eligible infants. For example, 11 (42.3%) of 26 cases became positive for *S. capitis* NRCS-A their first week in the NICU therefore would not have been eligible for a study investigating a two week exposure period. Matching reduced the number of non-colonised neonates who contributed control data. The small numbers and nested nature of the study resulted in repeated baseline and weekly data for individual neonates, some contributing as many as 26 weeks of control data. The neonates who contributed the most control data were those of low GA and low birthweight who were in the NICU for longer periods, therefore having more opportunity to match with cases in time. We used robust standard errors within the conditional logistic regression that would have accounted, to some extent, for the correlation between weeks of data provided by the same neonate. We were unable to perform a full multivariate analysis as our sample size was too small and would have produced unreliable results. The small sample size also precluded careful modelling of continuous variables, so there may also have been some residual confounding.

Conclusion

While the retrospective analysis had a number of limitations, on balance our results suggest that neonates requiring frequent contact due to more intensive medical management are at greater risk for *S. capitis* NRCS-A colonisation. If this is the case, the standard precautions for infection prevention and control used by the Dunedin Hospital NICU may not be adequate for preventing *S. capitis* NRCS-A colonisation. While the Dunedin Hospital NICU passes quarterly audits overall across five moments of hand hygiene, an audit focused on the individual moments of hand hygiene may be useful to determine whether individual moments are below compliance. Additionally, transmission-based contact precautions could be considered, as well as administrative measures such as cohorting neonates and ensuring staff who care for colonised neonates have no contact with non-colonised neonates. Our findings may be generalisable to NICUs that also have endemic *S. capitis* NRCS-A. A prospective study including staff member tracing to investigate *S. capitis* NRCS-A transmission is needed to further explore the findings of our study.

Author contribution

Louise M. Thorn: Software, Formal Analysis, Investigation, Data Curation, Writing — Original Draft, Visualisation, Project Administration.

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Conflicts of interest

All authors report no conflicts of interest relevant to this article.

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Appendix. Supplementary data

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