The ecology and antimicrobial resistance of Staphylococci colonising neonates

Heather Felgate*[1,2], Dheeraj Sethi[1,3], Gemma Langridge[1,2], Lisa Crossman[1,2,4], John Wain[1,2], Paul Clarke[2,3], Andrew Page[1], Mark A Webber[1,2]

Introduction

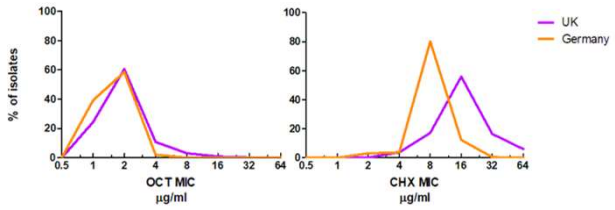
- Coagulase Negative Staphylococci (CoNS) are common skin commensals, accounting for ~20% of an infant's skin microbiome and help promote early innate immune responses (1-3).
- CoNS are however also opportunistic pathogens which can cause infection and are responsible for >50% of episodes of Late Onset Sepsis (LOS, sepsis after 72 hrs from birth) in neonates (4).
- LOS increases the length of hospital stay, invasive procedures, and provokes more and longer antibiotic treatments, which negatively impacts the long-term outcomes of new-born babies (1).
- Insertion of peripheral catheters occurs in almost all babies in neonatal intensive care units (NICUs) but is a major risk factor for infection as the skin barrier is breached. Antiseptics are critical to minimise infections.
- The emergence of antiseptic tolerance amongst CoNS is a major concern in the NICU and studies have identified isolates with reduced susceptibility associated with outbreaks of infection.
- Chlorhexidine (CHX) and Octenidine (OCT) are the most common agents used for skin antisepsis, however the mechanisms for resistance in CoNS are not very well understood.
- In this study we isolated CoNS from skin and rectal swabs from babies on NICUs from two countries (UK and Germany) where different antiseptic regimens were in place (5).

AIMS: To understand the population structures of isolates in NICUs and identify any genetic linkage to antimicrobial susceptibility.

Isolation of CoNS and antiseptic susceptibility

- Over 1000 isolates were characterised for antimicrobial susceptibility and sequenced.
- The most frequent species isolated were *S. epidermidis, S. haemolyticus* and *S. capitis* with similar strain types present in both units.
- Reduced susceptibility to CHX was observed in UK isolates (where CHX is used), compared to German isolates (where OCT is used). There was no change in susceptibility to OCT between the countries (**Figure 1**).

Figure 1. Susceptibility of all isolates to OCT and CHX.



Population structure of CoNS from NICUs

- Isolates were grouped using hierarchical Robust Clustering (RC) based on alignment of 16 ubiquitous *Staphylococcal* ribosomal proteins (7).
- This revealed a wide diversity of CoNS was present and allowed comparison of all species against each other (**Figure 2**), and AMR profiles varied by species (**Table 1**).
- *S. epidermidis* and *S. haemolyticus* were most common with heterogenous populations, *S. capitis* was also common but with a more clonal population structure.

Figure 2. Hierarchical Robust Clustering of CoNS NICU isolates visualised in iTOL (9).

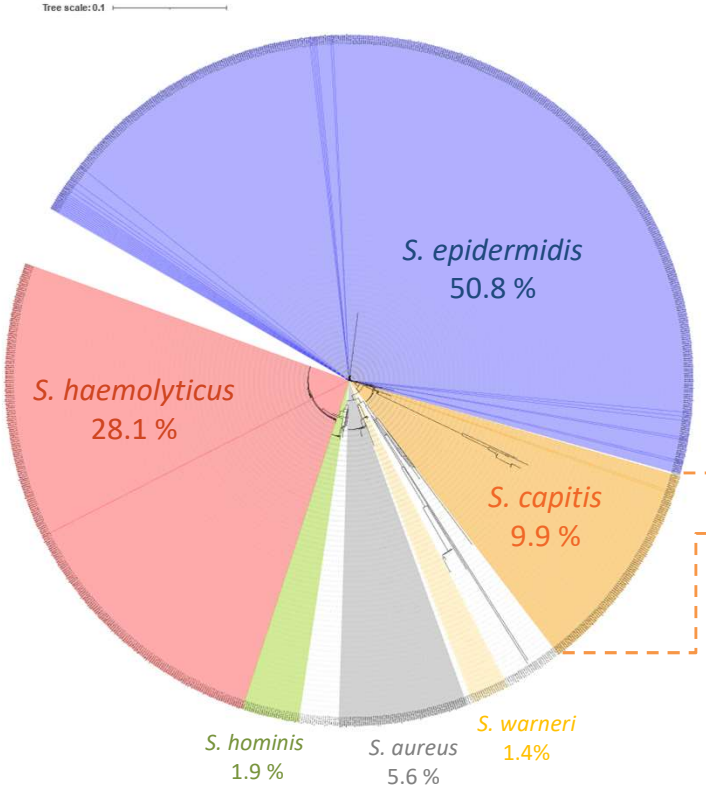


Table 1. Antimicrobial susceptibility of the NICU CoNS collection. Mean MICs for each species are shown (µg/ml) ; vancomycin (VAN), gentamicin (GEN), penicillin (PEN), cefotaxime (CEF), ciprofloxacin (CIP), daptomycin (DAP) and fusidic acid (FUS).

	OCT	CHX	VAN	GEN	PEN	CEF	CIP	DAP	FUS
S. aureus	2	**32**	1	8	2	4	0.5	0.25	2
S. capitis	2	**16**	1	12	4	4	0.5	0.25	**4**
S. epidermidis	2	**16**	1	8	2	4	0.5	0.25	2
S. haemolyticus	2	**16**	1	**16**	**64**	4	0.5	0.25	2
S. hominis	2	8	1	**20**	2	4	0.5	0.25	2
S. warneri	2	**16**	1	**32**	2	4	0.5	0.25	1

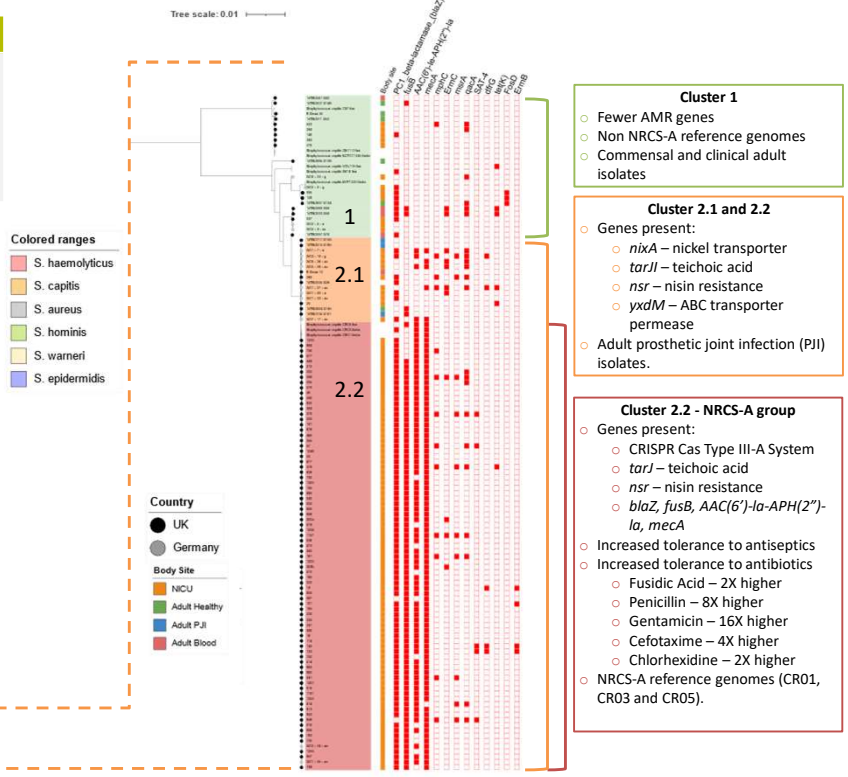
Staphylococcus capitis; a NICU adapted sub-clade

- A specific clone of *S. capitis* 'NRCS-A' has been identified worldwide as adapted to the NICU environment, and infection of neonates with this clone has been associated with higher morbidity and mortality rates than other CoNS (6).
- Using ROARY (8) core genome alignments distinguished the *S. capitis* isolates into three clusters (**Figure 3**). Previously sequenced isolates were included for context.
- The clusters were characterised as:

1. **Cluster 1:** Few AMR genes – mainly clinical isolates from adults.
2. **Cluster 2.1:** Common carriage of *qacA*.
3. **Cluster 2.2:** Many AMR genes – all NICU isolates and previously characterised NRCS-A strains (CR01 CR03 and CR05). This sub-cluster was characterised by:

- Increased MICs of multiple antibiotics and CHX.
- CRISPR Cas type-III system in an *SCCmec-cad/ars/cop* mobile genetic element.
- Presence of genes were involved in nickel transport and tryptophan synthesis.
- Absence of *btuD*, a Vitamin B12 importer was evident in isolates from this clade compared with other *S. capitis.*

Figure 3. Maximum Likelihood tree of *S. capitis* based on gene presence/absence from ROARY.



Conclusions

- A wide range of CoNS are carried by neonates on NICU and overlap with clinical isolates showing the skin is a reservoir for infection.
- These are similar in the UK and Germany and very similar strains of *S. capitis* are present in Germany and the UK *S. capitis* isolated from adults were however distinct from those from babies.
- The NRCS-A clone is commonly carried by neonates in NICUs but is split into two groups with a sub-clade (2.2) carrying more AMR genes and higher MICs to CHX and other antibiotics.
- No obvious reason for the CHX tolerance was seen from known AMR genes.
- This data provides new information about the phylogeny of CoNS in NICUs and suggests there are different potentials for selection of resistance between commonly used antiseptics.
- Increased tolerance to antibiotics and CHX differs between CoNS.

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Affiliations:
1. Quadram Institute Bioscience, Norwich Research Park, NR4 7UQ, Norwich, UK
2. Norwich Medical School, University of East Anglia (UEA), Norwich, UK
3. Norfolk and Norwich University Hospital (NNUH), NR4 7UY, Norwich, UK
4. SequenceAnalysis.co.uk, Norwich Research Park Innovation Centre, Norwich, NR4 7JG, United Kingdom

Author email:
*Heather.Felgate@quadram.ac.uk