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Analyzing staphylococcal contamination on surfaces and bedside areas of a neonatal intensive care unit of a children's hospital

Daniel See
University of Tennessee at Chattanooga, yvc226@mocs.utc.edu

Analyzing Staphylococcal Contamination on Surfaces and Bedside Areas of a Neonatal Intensive Care Unit of a Children's Hospital

Daniel Allen See

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Marissa McElrone, PhD, RDN	Henry G. Spratt, PhD	David Levine, PhD, DPT
Assistant Professor of Health and Human Performance	Professor of Biology, Geology, and Environmental Science	Professor and Walter M. Cline Chair of Excellence in Physical Therapy
Thesis Director	Thesis Examiner	Thesis Examiner

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ABSTRACT

Staphylococci species are known to be a cause of Healthcare-Associated Infections (HAIs) in neonatal intensive care units (NICU). There is limited research about the surveillance and identification of staphylococci bacteria from NICUs. Surveillance of bacteria within the NICU helps to identify areas acting as reservoirs for bacteria so that new cleaning policies and techniques can be put in place to stop the spread of HAIs. The objective of this study was to swab sample sites in a local level IV hospital NICU and identify locations of staphylococci presence throughout the NICU. Forty-one swabs were selected from over 900 swabs collected from the NICU at Erlanger Hospital for testing at the University of Tennessee Chattanooga's Clinical Infectious Disease Control lab. Using aseptic technique and standard microbiological procedures swab samples from the NICU were regrown as pure subcultures and tested using a variety of different tests, including the Remel RapID™ STAPH PLUS identification system to provide genus and species identities for the isolates. Of the 41 swabs selected, 17 different staphylococci species were observed, including *Staphylococcus aureus* and *Staphylococcus epidermidis.* The 17 different identified species were found on 45% of the swab sites throughout the NICU, with the most contaminated device being the suction yankauer (53%), and the highest contaminated surface being floors near sinks (41%). Further study for bacterial surveillance in the NICU will help to determine the best disinfection policies and cleaning practices for the decreased transmission of HAIs.

Section 1

Background and Introduction

1.1 Introduction

It is important for personnel working in healthcare settings to be aware of the potentially pathogenic bacterial flora present in their facility, and to be knowledgeable about disinfection options available to prevent infection of patients using their facility by the bacterial pathogens present. This is especially true for neonatal intensive care units, where neonates with conditions such as premature birth or underweight birth are more susceptible to Healthcare-Associated Infections (HAIs) resulting in increased risk for longer hospital stays, sepsis, or death. HAIs are defined as infections that patients did not have on admission but arise within 48 hours of admission to the facility. Staphylococci, particularly *Staphylococcus aureus*, is a leading cause of HAIs in the United States and one of the leading causes of serious infections in community settings (CDC, 2021). This study aims to examine the range of staphylococcal species present in a NICU healthcare setting, in addition to identifying frequent areas of bacterial contamination. By examining the bacterial species present and the areas of contamination, this study quantifies the amount of bacterial contamination present within the NICU and provides insight into the effectiveness of cleaning practices used in the NICU.

In this study, swab samples collected from the Erlanger Children's Hospital NICU were transported to the University of Tennessee at Chattanooga (UTC) Clinical Infectious Disease Control (CIDC) research lab to determine bacterial growth from the NICU samples. In the CIDC lab the swabs were used to inoculate selective and differential bacterial growth media specific to staphylococci. After incubation results from the agar plates were used to determine the areas of the NICU with the highest contamination of staphylococci. Verification subsampling from 15%

to 20% of the colonies that grew on the selective and differential growth media was used to identify the species of staphylococci present. This subsampling utilized the following tests: DNAse testing, coagulase testing, and RapID™ STAPH PLUS testing. The results of this testing resulted in identification of the major species of staphylococci that are problematic in NICUs: *Staphylococcus aureus* (SA) and *Staphylococcus epidermidis* (SE) (Bhatta et al., 2021). Data from a previous study of staphylococci in this NICU (Keilman et al., 2021) indicated that methicillin-resistant SA (MRSA) and methicillin-sensitive SA (MSSA) contamination rates were significant five years before this current study. To help guide the unit's Infection Preventionist and Medial Director develop plans to help reduce levels of infection by staphylococci that remain high in the unit, this study was requested. In addition to the benefits gained in Erlanger's NICU, the issue of NICU infant morbidity is a global topic, which expands the importance of studying staphylococci presence in this NICU.

Erlanger Children's Hospital has regularly tracked the presence of SA in the NICU population. Data from previous Erlanger bacterial identifications shows that SA colonization rates have stayed stable over long periods of time despite standard cleaning procedures. Beginning in 2015, the UTC CIDC research team partnered with Erlanger Health Systems to identify bacterial contaminants within Erlanger facilities. This included numerous projects to swab and identify bacteria in Hematology and Oncology units, NICUs, and out-patient clinics, and physical therapy clinics. Along with the UTC Physical Therapy program, the CIDC has worked to identify the various types of staphylococci bacteria present with the most common locations of bacteria in hopes to reduce bacterial spread. This study aims to quantify the amount of bacteria in areas of the Erlanger NICU by sampling to determine which sample areas have the highest amount of bacterial colonization. It was hypothesized that the rate of colonization per

1000 patient days will decrease with updated prevention, cleaning, and treatment procedures in areas found to have the most bacterial growth. The swab samples collected from the Erlanger NICU were inoculated in the UTC CIDC research unit to determine bacterial growth. The inoculation results from the CIDC lab determine the areas of the NICU with the highest contamination of general bacteria in addition to providing colonies to use for DNAse testing, coagulation testing, and RapID™ STAPH PLUS testing. The tests are run on pure cultures obtained from the originally incubated swabs and will identify SA, along with other types of staphylococci like SE, *S. capitis*, etc., that normally are associated with HAIs. With up-to-date data on specific bacterial growth, location, and frequency in the NICU, management of the unit will have new data to help avoid HAIs.

1.2 Literature Review

Infants admitted to a NICU are at a higher risk of developing HAIs due to a variety of factors: their underdeveloped immune systems, the high level of use of invasive devices to maintain life-supporting functions such as administration of medicine or food (Menezes et al., 2021). Also due to the extreme susceptibility of neonates to infections, drug-resistant microorganisms, such as methicillin-resistant SA (MRSA) play an even more important role in infections they develop. Gram-positive bacteria like staphylococci are frequently responsible for bloodstream infection in developed countries, with *Staphylococcus epidermidis* (SE) being one of the most common causes of bloodstream infections in neonates. Other infections in neonates are caused by Gram-negative bacteria, including *Klebsiella* species and *Pseudomonas* species (Goldstein et al., 2017). Menezes et al. (2021) suggest that admission of neonates with severe health conditions contributes to an overall increase in mortality rate in a NICU. Environmental

factors as a contributing factor in this increased mortality can't be ruled out. Many new studies provide evidence that additional surveillance of pathogen presence on environmental surfaces in NICUs need to be part control measures (Keilman et al., 2021; Bhatta et al., 2021; Menezes et al., 2021). Procedures for identifying possible bacterial growth in the NICU must be a priority and new cleaning practices must also be considered.

Bacterial contamination within a NICU is a major source of HAIs. Every year there are approximately one million neonate deaths worldwide, and in resource constrained countries up to 30%-40% of the deaths are due to HAIs (Bhatta et al, 2021). Bacteria, fungi, and viruses can contaminate NICU environment surfaces, sometimes surviving for days on surfaces (Keilman et al., 2021). In addition, colonized health care workers and patients with pathogens may also contribute to pathogen transmission (Bhatta et al., 2021). Attributing factors to pathogen transmission include poor hand hygiene, overcrowding, understaffing, inadequate training, and insufficient disinfection, leading to approximately one-third of HAIs being preventable by adapting stricter infection control techniques (Bhatta et al., 2021). Neonates within the NICU are most vulnerable to HAIs from contaminated objects, especially during surgical and mechanical manipulations like arterial and venous catheters (e.g., central lines), tracheal cannulas, shunt placement, and other procedures (Bhatta et al. 2021). A consideration to make when looking at the spread and location of bacterial contamination is the hygiene and disinfection policies in place in the NICU. A study that included employee phone-swabbing and a survey about phone use at work was conducted in a hospital setting and revealed that 30 out of 50 phones had viable bacterial present, with more than 19 phones containing four or more different species (Mark et al., 2014). Later in the survey portion of the study, 60% of participants revealed that they do not take time to disinfect their phone at work even though they "expect some sort of bacteria

present." The survey also revealed that some employees were reluctant to reveal phone use at work, with 25% responding with "no phone use" in one part of the survey, but 88% of survey participants answered a question regarding type of phone use. Regardless of whether the phone use is reported, cell phone use in clinics must be considered when making new cleaning and disinfecting policies. When NICU personnel are properly trained about stricter hygiene and disinfection policies, HAIs resulting conditions such as late-onset sepsis or bloodstream infections can be decreased.

The University of Tennessee at Chattanooga Clinical Infections Disease Control (CIDC) research team has previously studied bacterial contamination in clinical environments. Spratt et. al. (2014) conducted a study examining bacterial contamination of therapeutic ultrasound (US) device heads at nine southeastern Tennessee physical therapy clinics. Researchers swabbed patient-used US heads, US gel, and US gel bottles. After disinfection, the team re-swabbed the US heads to determine post-disinfection survival rate. Results showed significant amounts of nonspecific bacteria, SE, MSSA, and MRSA on gel bottle tips. A total of 52.7% of the gel bottle tip samples had viable bacterial contamination. Disinfection of the US heads resulted in substantial reduction of bacterial contamination (Spratt et al. 2014).

A research team at a different regional hospital analyzed the spatial and environmental factors in a NICU to compare areas of organism contamination and infection rates in the same NICU (Goldstein et al., 2017). Researchers discovered spatial correlation of increased neonate MRSA contamination risk depending on the colonization of neonate patients in neighboring pods. Neonatal MRSA contamination increased 1.5% due a clustering affect from medical equipment located throughout the pods. Bacterial identification such as *P. aeruginosa* (increased 2.6 times) and *K. pneumonia* (increased 30%) in addition to MRSA having increased

contamination odds in pods with more equipment space. The study found that in addition to extrinsic variables like equipment cluttering causing increased neonate patient colonization, factors such as characteristics of the infant and mother also contribute to increased bacterial presence. These factors are intrinsic and a focus of clinical management for infants. Inflammation or infections during pregnancy, labor, or delivery, combined with potential immature skin or under-developed immune system possibly affect the infant through direct contact with contaminated surfaces or indirectly with a visitor or healthcare worker. As Goldstein et al. (2017) summarize, the environment of the NICU is a reservoir for pathogenic and commensal organisms. Medical staff must consider that prevention of HAIs should focus on extrinsic and intrinsic factors within the NICU.

Controlling staphylococcal contamination in the NICU is a complex problem. There is sufficient evidence that shows conditions such as premature birth or underweight birth increase infant susceptibility to HAIs caused by staphylococci such as MRSA or MSSA. (Huang et al., 2006; Delaney et al., 2013; Milstone et al., 2020). Infection by MRSA or methicillin-sensitive SA (MSSA) can often be asymptomatic and puts immunocompromised or premature infants at risk for invasive infections possibly leading to mortality. Other staphylococci such as coagulase-negative staphylococci (CoNS) like SE and *S. capitis* have been shown to be significant threats to neonates due to resistances to antibiotics and antiseptics (Joubert et al., 2022; Laurent and Butin, 2019). CoNS infections are considered benign in most cases, but in low-weight or underdeveloped infants these infections can cause respiratory failure, lung disease, and death. The incidence rate of late-onset sepsis in preterm infants is most caused by coagulase-negative staphylococci (CoNS) with an 31%-54% (Berlak et al., 2018). However, both CoNS and coagulase-positive staphylococci (CoPS) must be targeted for prevention measures. Examples of

CoNS include SE, while an example of CoPS includes SA and MRSA. HAIs in the NICU, especially bloodstream-related infections, can frequently lead to sepsis due to indwelling devices and invasive procedures. While SE has been considered a main source of sepsis-associated CoNS in several studies, *S. capitis* has also been reported as a source of sepsis in several studies (Laurent and Butin, 2019; Butin et al., 2017; Ben Said et al., 2016; Carter et al., 2018). Laurent and Butin (2019) stress that *S. capitis is* an "unquestionably significant pathogen" in the NICU setting due to strains having resistances to antibiotics and antiseptics and because it may be responsible for serious infections in neonates. Similarly, two of the most common staphylococci bacteria found in a health care NICU setting, SE and SA, are potentially fatal in preterm and under-developed neonates. *S. epidermidis* and SA are also both dynamic bacteria that are constantly changing and evolving to resist antibodies and antiseptics (Joubert et al., 2022; Dong et al., 2018). With the constant presence of SE and SA, and the emergence of other CoNS bacteria like *S. capitis,* NICUs must be thoroughly inspected and regulated to make sure that proper disinfection and decolonization occurs.

A research group from New South Wales and the Australian Capital Territory combined eight NICU personnel teams to come up with stricter and more enforced cleaning and hygiene practices. By improving techniques for hygiene practices, including more strict hand washing, more extensive staff education, individualized bedside equipment, and submitting a monthly report of sepsis cases to senior clinical staff, researchers found that there was more than 50% decrease in infant sepsis from day 3 to day 35 (Bowen et al., 2017). The effectiveness in stricter and more extensive hygiene practices to decrease the spread of bacteria within the NICU is assisted when also knowing the common and locations of for bacterial environmental contamination on surfaces throughout the NICU.

A common theme in the studies noted above is the association of staphylococci bacteria with HAIs in neonates is that contamination of invasive devices is often linked with environmental factors in the NICU. Not only are these HAIs costly to the infants involved, but when the infections are deemed HAIs costs linked with the treatment of the patients falls on the hospital (CMS, 2018). Annually, HAIs account for $28 to $48 billion in costs per year for treatment of preventable infections, and within the NICU setting cost of care was almost $17 thousand more per infant with an HAI (Stone et al., 2009; Donovan et al., 2013). Interestingly, because of a perception that data from routine monitoring of hospital environmental surfaces did not correlate well with the incidence of HAIs, many hospitals reduced their surveillance sampling because of costs. In fact, during the 1970s both the US Centers for Disease Control (CDC) and the American Hospital Association discouraged the general monitoring of pathogens on surfaces in hospitals (CDC, 2019). As a result, between 1970 and 1975 it was estimated that 25% of hospitals in the U.S. reduced their routine environmental culturing (Haley and Shachtman, 1980). Eventually, the CDC established four conditions where environmental sampling would be warranted in hospital units. One such condition was to provide data in support of epidemiological studies focused on potential environmental links to the source of the pathogens. It is interesting that what 50 years ago was considered somewhat superfluous data on environmental contamination in hospitals has now changed when the hospitals are required to cover all costs associated with patient HAIs.

Because of the growing concern for environmental links to HAIs, hospitals spend large amounts of money to keep their facilities clean and disinfected. However, if improper or outdated cleaning practices allow bacteria contamination to go unnoticed the costs could he high. As done in this study, identifying the staphylococci present within the different environments of

the NICU provides necessary data to inform the unit's Infection Preventionist and Medical Director to update cleaning procedures as a means to decrease the number of HAIs reported in the NICU. The identification study presented here not only provided a complete list of staphylococcal species found over time at a level IV NICU, but also provided a map of hotspots showing locations of sites having high levels of bacterial contamination throughout the NICU that can be used for future HAI prevention efforts.

Section 2

Materials and Methods

This study was conducted in the Children's Hospital at Erlanger Neonatal Intensive Care Unit (NICU) through a collaborative agreement with unit's Medical Director. The research was conducted under the guidance of UTC faculty associated with UTC's Clinical Infectious Disease Control (CIDC) research group, in the CIDC lab in Hot Hall Rm. #305. To determine the baseline for the surveillance of Staphylococcal presence in the NICU, members of the CIDC in collaboration with the NICU Medical Director identified areas for Sway sampling listed in Table 1 within the Erlanger NICU as potential high contamination.

Table 1: Locations of initial swab sampling in the NICU. Of these sites the 10 most contaminated sites were sampled in Phase I of the study (see Table 2).

Individual Baby Isolettes	Rep	Communal Equipment	Rep	Physical Plant	Rep
Stethoscopes	7x	Bed Scales (Keyboard)	6x	Return Air Duct (HVAC)	7x
Electronic Thermometer	6x	Breast Milk Frig (Handle)	4x	Floor Near Sink	7x
Occupied Baby Bed (Handle)	6x	Breast Milk Freezer (Handle)	3x	Faucet	7x
Bath basins	6x	Blood Glucose (Keyboard)	6x		
Pump Controls	6x	Medication Pyxis (Keyboard)	1x		
Equipment Drawers	7x	Flip Bins	7x		
Computer Keyboard & Mouse	7x	Nurse Cell Phones	6x		
Nurse Cell Phones	7x	Breast Milk Warmer - Controls	7x		
Cardiac Monitor Screen	7x				

After initially swabbing all of the locations listed in Table 1 and determining the high contamination sample sites, the sampling regimen was reduced to include the 10 most contaminated sites in the pods (Table 2). Another aspect of the study was to divide our sampling into two phases: 1) sampling focused on common surfaces and items throughout the NICU pods, and 2) sampling focused on occupied infants' beds (isolettes). Sample locations and dates are listed in Table 2.

Table 2: Sample areas focused for swabbing during phase I and phase II

Phase I (3/1/21-6/7/21)	**Phase II (6/8/21-11/2/21)**
Stethoscopes	Occupied Baby Bed Handles
Occupied Baby Bed Handles	Head of Bed
Bath Basins	Foot of Bed
Bed Scale (Keyboard)	Port at Head
Flip Bins	Opening Port at Foot
Nurse Cell Phone	Water from Reservoir
Breast Milk Warmer Controls	Suction yankaeur
HVAC	Syringe Pump Controls
Sink Faucet	CR Monitor Controls
Floor Near Sink	Low Hanging Wires

The transport and swabbing process was identical to previous studies done by the CIDC in conjunction with Erlanger (Keilman et al., 2021). Sterile transport swabs (Fisherbrand single transport swabs) containing Stuart's medium were used to sample from the locations listed during both phases of sampling. For phase I samples were collected on 3/1/21 through 6/7/21, while for phase II samples were collected on dates 6/8/21 through 11/2/21. All totaled on any given sample date 70 swabs were collected. By the end of the study 957 swabs had been collected and analyzed.

To collect sample swabs each sample site was swabbed over an area of approximately 200 cm^2, being sure that the swab was rotated to ensure that the whole swab surface came into contact with the sample site. Swabs were assigned specific identification numbers recorded with

the date and sample site. After collection swabs were placed in a cooler on ice and taken to the CIDC laboratory for inoculation onto selective media within three hours of collection.

Inoculation occurred on two different types selective and differential media: Mannitol salt agar (MSA), aiding in detection of types of SA, and CHROM-MRSA agar, which is used to identify MRSA. Agar plates were divided into equal parts of either five or eight via Sharpie lines on the back of the plate. Numbers correlating to swab identification numbers were placed in each compartment in order to identify what swab site the inoculation represented. The swab was then inoculated in its designated plate compartment in a straight line. To inoculate the two types of media, the swabs would be rotated 180 degrees to bring another surface of the swab in contact with the second agar medium. All plates were incubated at 37 degrees Celsius for 48 hours.

After incubation, bacterial contamination from each swab was estimated by counting the colonies present on each plate. For the two different types of media colonial distinctions that are typically used to indicate either SA (on MSA plates) or MRSA (on CHROM MRSA plates) were used to identify the bacterial isolate (Keilman et al., 2021). As the numbers of colonies of the different distinct colonies were counted two scoring systems were used: the first was a simple presence or absence indication for each swab, the second was an estimation of the density of colonial growth present on the agar plates. The following ranking system was used to indicate the density of colonial growth from each swab in three categories: low contamination (1-5 CFU), moderate contamination (6-15 CFU), and high contamination (15+ CFU). Results as presence and density for all plate inoculations were recorded in Excel spreadsheets including swab number, number of colonies, and colonial morphology indicating potential MRSA, SA, or other staphylococci. After analysis of the growth on all the plates, subcultures were obtained from 15% to 20% of the colonies found growing on the selective and differential media. These

subcultures were aseptically transferred to a standard growth medium for staphylococci, Tryptic Soy Agar (TSA), and these cultures were incubated at 37 ^{o}C for 48 hours.

2.1 Materials and Preparation

Bacterial growth media used in this project that had to be prepared in the CIDC lab included: TSA slants and plates, Tryptic Soy Broth (TSB) tubes, and DNAase agar plates. Media that was purchased ready made for use in the CIDC lab included CHROM MRSA agar plates, purchased from Hardy Diagnostics (Santa Maria, CA), and Blood Agar plates with 5% sheep blood (Fisher Scientific, Waltham, MA) Other reagents necessary to run tests on the cultures were also made in the CIDC lab. As mentioned above TSA slants were used for the growth and identification of pure cultures. TSA was made by following manufacturer's instructions. The TSA solution was autoclaved for sterilization (15 PSI, 121^{o}C for 20 minutes). TSA slants were then made by transferring aliquots of TSA into tubes, autoclaving them, and then allowing them to cool on a slanted surface. Tryptic Soy Broth was made also by following the manufacturer's instructions. The TSB solution was transferred into test tubes with caps and then autoclaved for sterilization. A reagent necessary for the DNAase test, 5% hydrochloric acid was made in the CIDC lab by adding 10 mL of concentrated hydrochloric acid (12 N) to 200 mL of distilled water. Another reagent used for testing was the coagulase reagent, which was purchased from Fisher Scientific in dehydrated form, adding necessary water for rehydration prior to running the test. For specific identification of staphylococci, Remel RapID™ STAPH PLUS Identification Kits were purchased from Fisher Scientific.

2.2 Experimental Design

Depending on the type of test done, fresh samples of subcultures were required. After inoculation all media was then incubated for 24-48 hours at 37 ^{o}C. For the STAPH PLUS system the subcultures were first inoculated onto sheep blood agar plates, from which cells were aseptically harvested to inoculate the STAPH PLUS system. These tests were then incubated at 37 ^{o}C for four hours prior to reading the results. A total of 41 samples were selected for coagulase, DNAse, and STAPH PLUS testing, including one standard SA culture provided from the CIDC laboratory used as a control sample. Coagulase testing is a common test used for the identification of select staphylococci, which includes SA (Katz, 2010). For a coagulase test, 0.2 mL from a sample's freshly inoculated TSB was combined with 0.2 mL of Rabbit plasma and incubated at 37 ^{o}C for two hours. Throughout the two hours the sample was examined for clot formation, with samples containing clots marked positive and samples with no clot after the two-hour period marked negative.

Every sample that was used for a coagulase test was used for DNAse testing, which involves aseptically streaking DNAse plates with a loopfull of the sample culture using a line inoculation technique. After 24-48 hours of incubation at 37 ^{o}C, 5% HCl was added to the incubated DNAse plates. When hydrochloric acid is added to DNAse agar, it reacts with any intact DNA in the agar to make a cloudy, opaque agar. When the enzyme DNAse is produced by bacteria growing on the medium, and the DNA is broken down, because DNAse is an extracellular enzyme the enzyme diffuses out into the agar from the growth along the line inoculation breaking down DNA in its path. Then, when the HCl is added a "clear zone" with no cloudiness will be found surrounding the bacterial growth. For example, DNAse agar inoculated with a SA culture will have a defined area of clearing around the streak that does not turn cloudy

(See Figure 1). Possession of the DNAse enzyme is unique to SA, with CoNS staphylococci like SE being negative for DNAase, showing no clear zone near the bacterial growth (Figure 1). Coagulase testing, combined with DNAse plate testing, and MSA agar test results is a more efficient way to identify SA that any single method alone (Kateete et al., 2010).

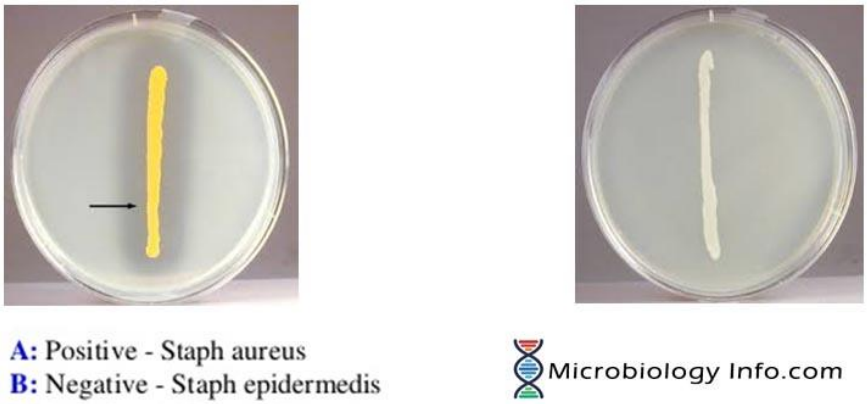


Figure 1

The last test performed on the cultures was the Remel RapID™ STAPH PLUS test (Figure 2), which can identify up to 40 species of staphylococci and select related genera. Following standard microbiological procedures, using colonies of the staphylococci growing on Blood Agar a turbid solution of mixed bacteria and RapID inoculation fluid was made using a

Figure 2

sterile pipette. This suspension was transferred via pipette into the test kits and incubated at 37 ^{o}C for four hours. Each test kit has 18 cavities, with each cavity containing a specific dehydrated reagent to detect properties of the bacterial suspension. Addition of the bacterial suspension to each of the cavities rehydrates the reagents in the cavity, allowing the

bacteria to cause some form of reaction to occur in the cavity. The reactivity of each cavity is then scored and compared to reactivity patterns of various staphylococci recorded in the ERIC computerized database.

The ERIC database works exclusively with Remel to provide probability percentages, bioscores, contraindicated results, accessory tests, and clinically relevant commentary. ERIC data was then stored in an Excel table to compare various species of staphylococci, quantity per species, location within the NICU, and original CFU details from after swab inoculation.

Section 3. RESULTS

3.1 Total Data

Table 3: Results generated using the Remel RapID™ STAPH PLUS test on the cultures tested. Species identities determined using the Remel ERIC database.

	ERIC-Derived Suspected Organis	Probability %	Location
SA control	S. aureus	>99.9%	
CHR 749	S. aureus	99.89%	Head of Bed Pod 5
CHR 662	S. aureus	>99.9%	Suction Yankauer Pod 1
CHR 762	S. auricularis	83.07%	Suction Yankauer Pod 6
MSA 276	S. caprae	98.18%	Floor Near Sink Pod 3
CHR 329	S. capitis ss capitis	99.56%	Floor Near Sink Pod 2
CHR 286	S. capitis ss capitis	>99.9%	Floor Near Sink Pod 4
CHR 954(y	S. capitis ss capitis	99.36%	Suction Yankauer Pod 6
**MSA 864 **	S. capitis ss urealyticus	>99.9%	Suction Yankauer Pod 2
CHR 419	S. chromogenes	95.82%	Occupied Baby Bed Handle Pod 5
MSA 864 1	S. cohnii ss cohnii	>99.9%	Suction Yankauer Pod 2
**MSA 864 **	S. cohnii ss cohnii	96.49%	Suction Yankauer Pod 2
CHR 308B	S. cohnii ss cohnii	97.05%	Sink Faucet Pod 7
CHR 817	S. cohnii ss cohnii	97.43%	Suction Yankauer Pod 5
MSA 447	S. cohnii ss cohnii	>99.9%	Floor Near Sink Pod 7
CHR 211	S. cohnii ss cohnii	>99.9%	Floor Near Sink Pod 3
CHR 368C	S. cohnii ss urealyticus	>99.9%	Floor Near Sink Pod 6
CHR 14a	S. epidermidis	>99.9%	Floor Near Sink Pod 1
MSA 378	S. epidermidis	99.52%	Floor Near Sink Pod 7
MSA 317	S. epidermidis	99.87%	Return Air Duct Pod 1
MSA 378	S. epidermidis	99.52%	Floor Near Sink Pod 7
CHR 408	S. epidermidis	>99.9%	Floor Near Sink Pod 3
CHR 255B	S. epidermidis	99.80%	Floor Near Sink Pod 1
CHR 936	S. epidermidis	99.15%	Suction Yankauer Pod 4
MSA 149	S. epidermidis	98.34%	Floor Near Sink Pod 3
MSA 159	S. epidermidis	98.34%	Floor Near Sink Pod 4
MSA 797	S. epidermidis	98.93%	Port at Head w/ object Pod 2
CHR 871	S. epidermidis	90.03%	Port at Head w/ object Pod 4
CHR 149	S. galinarum	>99.9%	Floor Near Sink Pod 3
CHR 671	S. hyicus	>99.9%	Suction Yankauer Pod 2
CHR 308A	S. hyicus	99.65%	Sink Faucet Pod 7
CHR 148	S. hyicus	99.65%	Sink Faucet Pod 3
CHR 129B	S. hyicus	>99.9%	Nurse Cell Phone Pod 1
CHR 660	S. pasteuri	96.86%	Port at Foot Pod 1
**MSA 954 **	S. saprophyticus	> 99.9%	Suction Yankauer Pod 6
CHR 744	S. lugdunensis	99.19%	Suction Yankauer Pod 4
CHR 419	S. vitulinus	>99.9%	Occupied Baby Bed Handle Pod 5
MSA 100A	S. warneri	96.29%	Floor Near Sink Pod 1
MSA 408	S. warneri	96.29%	Floor Near Sink Pod 3
CHR 129A	S. xylosus	>99.9%	Nurse Cell Phone Pod 1
MSA 45	N/A		Bed Scale Keyboard Pod 3

Table 4: Results of DNAase and Coagulase tests on cultres tested. Additionally, colonial morphology of the isolates also presented.

	DNAse	Coagulase	Colonial Morphology
CHR 286	Positive	Negative	3/ wt 1MM
CHR 817	Negative	Positive	3 wt-blue <1MM
CHR 749	Positive	Positive	Wt-pink <1MM
MSA 408	Positive	Positive	3/ wt 1MM (yel)
CHR 954	Positive	Negative	TNTC/ Wt 1MM
CHR 129B	Negative	Negative	1/ pink 1MM
CHR 936	Positive	Negative	Purple 1MM
CHR 671	Positive	Positive	29/ Wt-pink 2MM
CHR 368C	Positive	Positive	1/ blue 3MM
CHR 660	Positive	Positive	28/ Purple 1-2 MM
CHR 308A	Positive	Positive	2/ blue 2-4MM
CHR 148	Negative	Positive	1/ wt-pink 3MM
MSA 378	Positive	Positive	6/Wt 1-2 MM (Yel)
CHR 419	Positive	Positive	1/ wt 1MM
SA control	Positive	Positive	
MSA 447	Positive	Positive	2/ purp 1MM; 4/pink 1MM
CHR 308B	Negative	Negative	5/ pink-purple 2-3 MM
CHR 871	Positive	Positive	1/ Tan-Wt 3MM
MSA 276	Positive	Negative	60+/ wt 1MM
CHR 408	Positive	Positive	1/ Wt-pink 1MM
CHR 149	Positive	Positive	1/ tan-purple 2MM
MSA 45	Positive	Negative	2/wt 2MM (yel)
CHR 662	Positives	Positive	TNTC/ Purple 1-2 MM
CHR 329	Negative	Negative	1/wt 2MM; 3/wt 1MM
MSA 378	Positive	Positive	6/ Wt 1-2 MM (Yel)
MSA 864 Tan	Positive	Positive	TNTC/ Wt 1MM (yel)
MSA 149	Positive	Positive	29/ wt 1-2MM
CHR 762	positive	Positive	TNTC Wt-pink 1MM
CHR 419	Positive	Positive	1/ wt 1MM
MSA 864 Yel	Positive	Negative	TNTC/ Wt 1MM (yel)
MSA 317	Positive	Negative	2/ wt 2-3MM (red)
CHR 744	Positive	Positive	TNTC/ Purple 1MM
CHR 129A	Positive	Negative	1/ blue 2MM
MSA 100A	Negative	Negative	17/ wt 2MM (yel)
CHR 211	Negative	Negative	7/ pink 1MM
CHR 14A	Negative	Negative	3 pink-purple 1MM
CHR 255B	Negative	Negative	6/ wt-pink 1-2 MM
MSA 797	Negative	Negative	3 wt, 2MM (red)
MSA 954 Yel	Positive	Positive	TNTC/ Wt 1MM (yel)
MSA 159	Positive	Positive	4/ wt 2-4MM (yel)

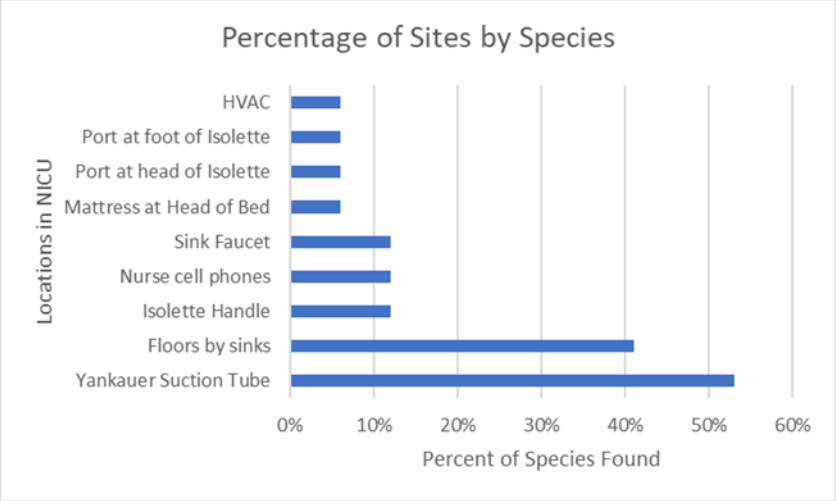
3.2 Descriptive Results

Of the 957 swabs collected, 41 were chosen to be sub-cultured and tested for species identification. In addition, one sample of SA provided from the CIDC laboratory was sub-cultured and used as a control for the Remel RapID™ test kits. For the swabs 38 of the 41 gave >95% confidence for identification, two swabs had 80%-90% confidence for identification, and one swab had <80% confidence. Of the 41 samples sub-cultured and tested there were 17

different staphylococci species identified (Table 3). The most common species identified was SE with 11 identifications, followed by *Staphylococcus cohnii subspecies cohnii* with six identifications and *Staphylococcus hyicus* with four identifications. There were four identifications of *S. capitis*, which includes both *S. capitis* subspecies capitis and *S. capitis* subspecies urealyticus, SA had two identifications from the Remel RapID™ test kit.

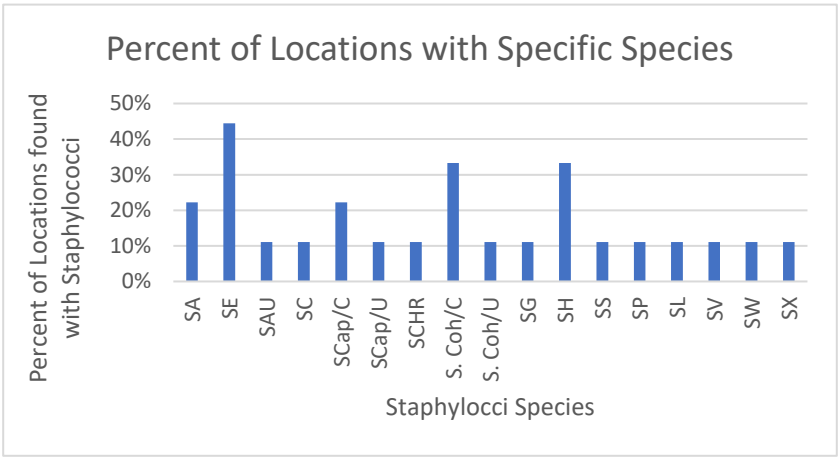
Contamination of the sample swab locations by staphylococci tested throughout the NICU varied. The most consistently contaminated site was the suction yankauer with 53% of staphylococci species identified, followed by the floors near sinks with 41% (Graph 1). Staphylococci presence dropped in other contaminated areas within the NICU, with 78% of the NICU sites having low contamination.

Graph 1: Percentage of staphylococci species observed on swabs from sites.



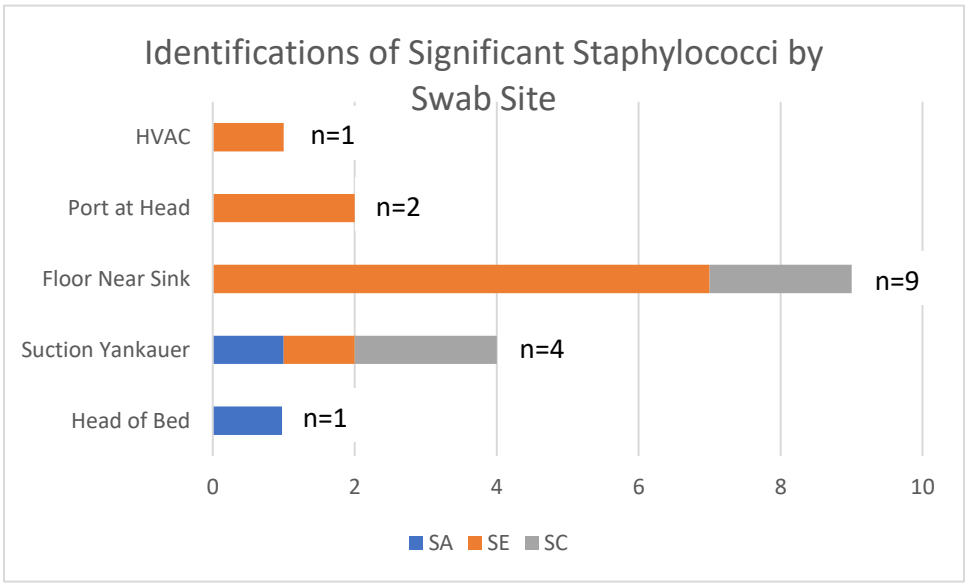
Mannitol-negative staphylococci contamination was most common, with SE being found at 44% of sites with contamination. SA and SC were both found at 22% of sites with contamination. Other notable species include *S. hyicus and S. cohnii*, which were found at 33% of contaminated sites (Graph 2).

Graph 2: Percent of Locations with Specific Species



SA = S. aureus
SE = S. epidermidis
SAU = S. auricularis
S. Cap/C = S. capitis ss capitis
S. Cap/U = S. cohnii ss urealyticus
SG = S. galinarum
SC = S. Caprae
S CHR = S. Chromoganes
S. Coh/C = S. cohnii ss cohnii
S. Coh/U = S. cohnii ss urealyticus
SH = S. hyicus
SS = S. saprophyticus
SP = S. pasteuri
SL = S. lugdunensis
SV= S. vitulinus
SW= S. warneri
SX= S. xylosus

Graph 3: Significant Staphylococci species by swab site (n = number of swab samples).



Section 4

Discussion

This study has demonstrated the presence of and further identified Staphylococci bacteria on select environmental surfaces in the Erlanger NICU. Overall, 45% of NICU sites swabbed had Staphylococci, with SA identified at 22% of the contaminated sites. The findings from this study provide evidence of Staphylococci contamination that could contribute directly to HAIs in neonates. A growing number of studies suggest that environmental contamination may be linked to HAIs, but fewer studies have addressed environmental contamination in pediatric settings (Keilman et al., 2021). A study of an Italian NICU provided data showing the most contaminated sites, including scales, bedside equipment, bed isolette ports, health care workers' phones, and computers, among others (Auriti et al., 2003). Some of the contaminated sites found in this study included objects or areas listed by Auriti et al. like cell phones, bed isolettes, or bedside equipment such as the consistently contaminated suction yankauer. Hospital floors as possible bacteria reservoirs have also been a consistently contaminated site for close to half of a decade. For the NICU studied here the floor area by the sink in each pod was the second highest consistently contaminated area within the NICU and may serve as a reservoir for Staphylococci contamination in the unit.

Of the 41 swabs sub-cultured, 17 Staphylococci species were identified, with multiple swabs being identified for SA and SE. A surprising number of sites swabbed were found to have *S. hyicus and S. cohnii,* as well as multiple swabs identified as *S. capitits* present. *S. capitis* bacteria has been the subject of study as possible source of HAIs in neonates and is bacteria normally found in regions of the head following puberty with occasional isolates from blood, urinary tract, joints, and wounds (Remel ERIC, 2002). The effect of *S. capitis* on HAIs in neonates is still not well studied. There are some studies that link increased HAIs in neonates to *S. capitis*. Further research is required to determine the full threat of *S. capitis* to neonates.

Similarly, *S. hyicus* and *S. cohnii* species are uncommon in literature focused on bacterial contamination in NICUs, and their relatively high levels of identification in this study suggests that further investigation of their importance in this unit. *S. hyicus* is a bacterium important in veterinary infections of domestic animals and livestock, while *S. cohnii* is a bacterium involved in the formation of arthritis processes and is only occasionally isolated in clinical environments (Remel ERIC, 2021). The presence of high numbers of unusual Staphylococci, as well as the variety of Staphylococci species present may also require further study to determine possible links to HAIs in neonates.

There is also a discrepancy between traditional methods used for identifying SA (Mannitol Salt Agar test combined with coagulase and DNAse testing) and the Remel RapID™ identification of SA. According to the tests conducted here, there were 6 swabs that had positive growth for MSA (yellow tint to the agar around colony formation) while also testing positive in the coagulase and DNAse tests. Coagulase testing in conjunction with MSA and DNAse testing is an ideal method for the identification of SA, especially in areas with limited resources or income (Kateete, 2010). However, of these 6 MSA samples that tested coagulase and DNAse positive, none were identified as SA using the Remel RapID™ system. There is limited data on the use of Remel RapID™ as a regular surveillance tool in the NICU and the use of a computerized system to determine the identity of bacteria needs to be looked into further.

The spread of staphylococci throughout the Erlanger NICU was similar in a previous study of staphylococci in this same NICU in 2015 and 2016. In Keilmen et al.'s (2021) study, the most contaminated sites in the unit were the return air duct and the floors near the sink. In this study, conducted in 2021 the site with the highest levels of staphylococci contamination were the bedside suction yankauers and the floors near the sinks. Similar to the previous study six years

earlier, a common contamination site continues to be the floor area around the sinks. As per multiple studies, invasive devices and bedside equipment are two leading sites of staphylococcal contamination within NICUs (Menezes et al., 2021; Bowen et al., 2017). The rest of the identified staphylococci samples were typically found in areas having concentrations of equipment or in areas of high foot traffic. The possibility of staphylococci bacteria being spread throughout the NICU by patients, visitors, and healthcare workers is a serious threat due to the important and highly populated areas currently showing contamination, particularly in light of the high levels of staphylococcal contamination of the unit floors. The layout and traffic patterns of the NICU may need to be assessed if routine and revamped cleaning procedures do not have an impact on staphylococci levels.

Previous studies have shown the effectiveness of stricter and more enforced cleaning and hygiene policies in decreasing the number of reported HAIs in NICUs. Bowen et al. (2017) showed a decrease in sepsis due to CoNS with stricter personal hygiene practices, increased staff knowledge, personalized bedside equipment, and monthly reports of HAIs to supervisors. A similar effort focusing on controlling MRSA outbreak and spread from NICUs in the Chicago area found that in addition to the standard hand-washing procedures and patient isolation, bacterial culture surveillance in the NICU could have an increased effect on staphylococci spread (Gerber et al., 2006). The one thing that is certain is that with routine surveillance and identification of bacteria within the NICU the most effective policies should be put in place to prevent and treat HAIs.

Section 5

Limitations and Future Research

This study has provided evidence that staphylococcal bacteria are capable of surviving on environmental surfaces in a NICU. High levels of contamination were found on the suction yankauer and on the floor near the sinks, with relatively low levels of contamination present throughout other parts of the NICU. Despite the clear presence of staphylococci within the NICU, further surveillance of bacteria in the NICU is a necessity part of a plan to help reduce bacterial contamination in the unit. Other parts of this plan should include education of unit personnel to reduce person-to-person spread of pathogens, and to use the most up to date cleaining/disinfecting protocols possible. Use of the Remel RapID™ STAPH PLUS system in a microbiology lab studying swabs collected from the unit can help in this process by offering the benefit of a supporting database for staphylococci identification. Future work with the Remel RapID™ STAPH PLUS system database is needed to confirm database accuracy when compared with different laboratory methods for identifying bacteria such as SA or MRSA.

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