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Methicillin-Susceptible Staphylococcus aureus Colonization in the Neonatal Intensive Care Unit

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Colonization in the Neonatal Intensive Care Unit

A Thesis Submitted to the

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Shoaib Syed Ahmed

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METHICILLIN-SUSCEPTIBLE *STAPHYLOCOCCUS AUREUS* COLONIZATION IN THE NEONATAL INTENSIVE CARE UNIT

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Introduction:

Nasal carriage of *Staphylococcus aureus* is a sensitive indicator of staphylococcal colonization and is considered a source of subsequent infection. When the incidence of *Staphylococcus aureus* colonization increased in our neonatal intensive care unit (NICU), resulting in 3 methicillin-resistant *Staphylococcus aureus* (MRSA) infections over a 3 month period, we sought to further our understanding of *S. aureus* epidemiology and response to infection control practice. The purpose of this investigation was to study the methicillin-susceptible *Staphylococcus aureus* (MSSA), and further determine the clonal spread of MSSA strain types. Since few studies have analyzed the clinical profile of MSSA in neonates, we hypothesized that the incidence of MSSA colonization would follow a mixed endemic and epidemic pattern over the period of the study. We further compared the MSSA colonization data to that of MRSA, in order to get a fuller picture of the circulating *S. aureus* strain pool in the NICU.

Methods:

This retrospective longitudinal study consisted of infants hospitalized in a Level III-IV NICU (approximately 45 beds) from April 2003 to December 2004. Nasal surveillance cultures of all infants were obtained on admission and weekly and pulsed-field gel

electrophoresis (PFGE) was used to determine *Staphylococcus aureus* strain type. Transmission of identical strains among infants was noted. By testing for antibiotic susceptibilities, the prevalence and transmission data of MSSA was calculated. Thereafter, epidemiologic data such as birth weight, age, therapeutic modalities, length of admission, and antibiotic use was obtained from clinical summaries and used to characterize patients who had been colonized with MSSA.

Results:

During the 21 month study period, 1081 infants were screened for *S. aureus*. Of these, 877 (81.1%) tested negative, and 156 (14.4%) tested positive for MSSA. The prevalence of colonization with MSSA approached 45% by the 5th week of hospitalization for any given infant, and 70% in 9 weeks. Following the institution of routine nasal surveillance in April 2003, the incidence of MSSA cases fell from 6.5 to 1.5 per 1000 patient-days per month. Molecular typing using PFGE demonstrated three prevalent MSSA clones: clone "4" (7%), clone "15" (11%), and clone "23" (12%), corresponding to periods of increased incidence. The median length of stay was significantly longer in the intensive care infants compared to the continuing care i.e. "feed-and-grow" infants (median 77 versus 44 days, wilcoxon p=0.05). The mean length of stay was also longer in the intensive care infants, although this did not reach statistical significance (86 versus 66 days, student's t-test p=0.18). On chi square analysis infants in intensive care rooms were found to have a significantly higher prevalence of MSSA isolates than continuing care rooms in the nursery (54% vs. 35% of the total pool, $P < 0.04$). By comparison, 48 (4.4%) infants tested positive for MRSA, and the incidence of MRSA cases fell from 5.8 to 0.4 per 1000

patient-days per month during the study period. One predominant MRSA clone, clone "9", was identified and controlled.

Conclusions:

Control of MSSA is challenging because colonization is expected, endemic infections are tolerated and surveillance usually focuses on drug-resistant pathogens. During the period of study for this critically ill infant population, the incidence of both MSSA and MRSA colonization fell dramatically in response to the reinforcement of hand hygiene and contact precautions. We also identified an increased risk for MSSA colonization among intensive care infants as compared to continuing care infants, which is most likely connected to the significantly longer hospital stays among intensive care infants. The findings emphasize the need for cost-effective surveillance strategies for endemic infections in order to monitor progression from colonization. Finally, knowledge of the pattern of healthcare associated infection can contribute to the intensification of infection control measures and the updating of antibiotic usage guidelines.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

I. BACKGROUND

Epidemiology and Infection control

Infection control and hospital epidemiology are concerned with detection and preventing the spread of infections within the health-care environment. Hospital epidemiology is practiced within the confines of a particular health-care delivery system rather than directed at society as a whole. Infection control concerns itself with prevention (hand hygiene, disinfection, sterilization, vaccination, and surveillance), investigation and management of demonstrated or suspected spread of infection within a particular health-care setting. In many institutions, routinely collected statistics for the purposes of surveillance are an essential way of describing the occurrence and magnitude of disease, monitoring morbidity and mortality, and creating a data base for pinpointing risk factors that can then be avoided.. As such, it is a practical sub-discipline of epidemiology that is often under-recognized and under-supported even though it is an essential part of the infrastructure of good health care delivery (1).

Staphylococcus aureus as a human pathogen

Staphylococci are ubiquitous in the environment. The most common cause of staphylococcal infections, *Staphylococcus aureus*, is a spherical bacterium that can cause a range of illnesses from minor skin infections and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis, toxic shock syndrome, and septicemia (2).

A Gram-positive coccus, it appears as grape-like clusters when viewed through a microscope and as large, round, golden-yellow colonies, often with β-hemolysis, when grown on blood agar plates. The golden appearance is the etymological root of the bacteria's name: aureus means "gold" in Latin.

S. aureus is catalase positive and thus able to convert hydrogen peroxide to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci. *S. aureus* can be differentiated from most other staphylococci by the coagulase test: *S. aureus* is coagulase-positive, while most other staphylococcus species are coagulase-negative. *S. aureus* has about 2,600 genes and 2.8 million bp of DNA in its chromosome (3).

Humans are a natural reservoir for *S. aureus* and asymptomatic colonization is far more common than infection. As a commensal on human skin, colonization of the nasopharynx, perineum, or skin (especially if cutaneous disruption or damage is present) may occur shortly after birth and may recur anytime thereafter (4). Family members of a colonized infant may also become colonized. Transmission occurs by direct contact with a colonized carrier. Carriage rates are reported to vary greatly; notably, higher rates than in the general population have been documented in patients in intensive care units, patients with long-term indwelling intravascular catheters, and health-care workers. Young children tend to have higher colonization rates, possibly because of their frequent contact with respiratory secretions (5). Colonization may be transient or persistent and can last for years. The finding of *S. aureus* under these circumstances does not always

indicate infection and therefore does not always require treatment (indeed, treatment may be ineffective and re-colonization may occur). However, in infants *S. aureus* infection can cause a severe disease known as staphylococcal scalded skin syndrome; deeply situated *S. aureus* infections can lead to bacteremia, staphylococcal endocarditis, and pneumonia, which may be rapidly fatal.

Approximately 30% of healthy adults carry *Staphylococcus aureus* on their skin or in their anterior nares at any given time (3). Worldwide, an estimated 2 billion people carry some form of *S. aureus*; of these, up to 53 million (2.7%) are thought to carry MRSA. In the United States, 95 million carry *S. aureus* in their noses; of these 2.5 million (2.6%) carry MRSA (6). A population review conducted in 3 communities in the US showed the annual incidence of community-associated MRSA during 2001–2002 to be 18–25.7/100,000; most isolates were associated with clinically relevant infections, and 23% of patients required hospitalization (7).

Colonization and Infection in the Nursery

Newborn infants are initially devoid of endogenous flora and thus are more prone to bacterial colonization with environmental organisms while in the neonatal intensive care unit (NICU). The 2 major modes of acquisition are the perinatal transfer of maternal vaginal flora to the infant (vertical transmission) and acquisition after birth from an environmental or human source (horizontal transmission). With the survival of an increasing number of premature infants, the average hospital stay of infants in the NICU

has been longer; thus, the opportunity for acquiring micro-organisms while in the NICU environment is greater.

Healthcare associated infections are defined as infections that manifest 48 hours after admission to the neonatal intensive care unit (8). However, the incubation period of neonatal infections differs and some perinatally acquired infections are known to manifest after 48 hours of life, especially those with maternal predisposing factors for sepsis such as chorioamnionitis, premature rupture of membranes or maternal infections. Fonseca et al (9) extensively reviewed the literature on healthcare associated *S. aureus,* with emphasis on the methodological soundness of studies, and classified them by applying standard epidemiological criteria of quality. They found that few studies concerning endemic rates of healthcare associated infections give an overall picture of the problem in the newborn population, a problem all the more acute since *S. aureus* and coagulase negative staphylococci (CNS) have now replaced gram-negative bacteria as the most common etiological agents. Skin infections, pneumonia and bacteremia were the most common healthcare associated infections for infants. Risk factors included prolonged hospital stay, very low birthweight (<1500 g) and invasive procedures such as endotracheal intubation, hyperalimentation, or intravenous feeding. The question of the *most* significant risk factor for the neonatal acquisition of a healthcare associated infection was not satisfactorily answerable. However, the most reliable and reproducible studies with statistical analysis documented that mothers are *not* an important source of *S. aureus* for their infants; that nursery personnel play a minor role whether as reservoir or source in outbreak situations; that other infants, once the pathogen has been introduced

are *themselves* the reservoir for sustaining an outbreak, the primary localization being the nose or umbilicus; that the ultimate origin of new pathogenic strains is still uncertain but some evidence points to the health care personnel; that the environment is not the source of *S. aureus* for the infants; that transmission is usually mechanical, on the hands of health care personnel; that colonization often precedes the development of disease, and finally that high rates of colonization may exist without the occurrence of an outbreak. They also found that prophylactic or non-specific topical use of antibiotics is not advisable and that monitoring the rate of overt infection is more efficacious than monitoring the rate of colonization.

Investigations of *Staphylococcus aureus* in the 1950s and 1960s in both longitudinal and epidemic situations provided the first solid data regarding the epidemiology of colonization and disease among newborn infants. Studies were designed to evaluate a variety of other factors which might influence colonization rates, including the use of central observation nurseries, cohort nurseries, antibacterial agents on the infant's nares or umbilical cords, and the colonization rates of mothers and personnel. Many of these factors appeared to be interacting at different times (10).

Since many neonates are colonized within the first week of life, 20-30% of normal infants carry at least one strain of *S. aureus* in the anterior nares. The organisms may be transmitted from the nose to the skin, where colonization appears to be more transient. Repeated recovery of *S. aureus* from the skin would suggest repeated transfer rather than persistent skin colonization. However, persistent umbilical and perianal carriage occurs.

Transmission of *S. aureus* generally occurs by direct contact or by the spread of heavy particles over a distance of < 2m. Heavily colonized individual carriers are particularly effective disseminators. Autoinfection is thought to be common, and minor infections such as pustules, paronychia and styes may seed more disseminated infections. Consistent hand hygiene between patient contacts decreases the spread of staphylococci from patient to patient. Incidentally, older children and adults are more resistant than neonates to colonization (11).

Invasive disease may follow colonization. Antibiotic therapy with a drug to which *S. aureus* is resistant favors colonization and the development of infection. The antibiotics most commonly used empirically in the nursery are ampicillin and gentamicin. Third-generation cephalosporins are restricted and are mainly used for treating infections that are the result of antibiotic-resistant organisms and for treating meningitis. Other factors that increase the likelihood of infection include wounds, skin disease, ventriculo-peritoneal shunts, intrathecal or intravenous catheterization, corticosteroid treatment, malnutrition , acidosis and azotemia. Viral infections of the respiratory tract may also predispose to secondary bacterial infection with staphylococci (11).

In one study, rates of colonization by *Staphylococcus aureus* were determined for 9515 infants admitted to the nursery of a general hospital during a six-year period (12). The mean colonization rate was 14%, but there were no consistent phage types present or changes in the number of phage-typable staphylococci during this period. However, during one year of this study a certain phage type became endemic and accounted for

74% of colonization due to *S. aureus*. Anterior nares cultures of the mothers and nursing personnel, umbilical cord cultures of the infants at the time of admission, of the bath water following admission bathing, and of fomites within the nursery excluded these potential reservoirs as the cause of the high colonization rates. The primary reservoir of staphylococci was apparently the infants themselves and the postulated method of spread was hand carriage by healthcare workers and transmission of the organism from infant to infant.

In another study, measuring umbilical colonization rates for *S. aureus* among the total infant population yielded a mean colonization rate of 18% over a six-year period. The data were analyzed by the use of simple analysis of variance. There was no significant change in the mean *S. aureus* colonization rate of umbilical cords following the discontinuation of hexachlorophene midway through the study period (19).

Methicillin-resistant Staphylococcus aureus (MRSA)

During the last decade, there has been an increase in the proportion of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in both inpatients and outpatients, and especially among infants in neonatal intensive care units. This has become a major concern because of the higher mortality due to systemic MRSA infections. Serious staphylococcal infections may lead to dire consequences, especially with regard to therapy with antimicrobial agents, since they have gradually evolved resistance to most classes of antibiotics. Already over 95% of patients with staphylococcal infections,

worldwide, do not respond to therapy with penicillin or ampicillin (13). Reported for the first time in the 1960s, MRSA became ever more prevalent in the 1980s and is currently endemic in various healthcare settings. A large study of Australian hospitals over the last 30 years concluded that infected and colonized patients were the primary reservoirs of organisms, and that transmission was mainly via hospital staff (14). MRSA strains are thought to have evolved by acquisition of a genetic element named staphylococcal chromosomal cassette (SCC) mec, which carries the mobile mecA gene. Acquisition of the mecA gene codes for an altered penicillin-binding protein (PBP) that has a lower affinity for binding β-lactams (e.g. penicillins, cephalosporins and carbapenems). This confers resistance to all β-lactam antibiotics and obviates their clinical use during MRSA infections. The mechanism responsible for mecA transfer is not known, but evidence supports horizontal transfer of the mecA gene between different staphylococcal species (15) as well as different gram-positive bacteria. Five different types of SCCmec, which differ in size and structure, have been described for *S. aureus* (16).

The β-lactamase-resistant penicillins (i.e., methicillin, oxacillin, cloxacillin and flucloxacillin) were developed to treat penicillin-resistant *S. aureus* and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used (it was introduced in 1959), but only two years later, the first case of MRSA was reported in England. Though not uncommon in hospitals during the 1980s, MRSA rates increased even more in the 1990s, when there was an explosion in prevalence and it became endemic. In Brazil Sader et al., after analyzing 30 samples of MRSA isolated from nine hospitals in the city of São Paulo, observed the predominance of an endemic clone, found

in eight of the nine hospitals enrolled in the study (34). In Japan, the neonatal MRSA carrier rates are relatively high, reaching between 40 and 60% of total neonates in the NICU. The presence of MRSA in nose, pharynx, or umbilicus was identified was a significant risk factor for the development of invasive MRSA infections. Outbreaks were variously attributed to intravenous catheters, the use of generalized or topical antimicrobials, low birthweight babies, prolonged hospital stays, untrained staff, overcrowding, limited space and understaffing (38, 39).

Topical mupirocin (pseudomonic acid) has been used to try to eradicate nasal and skin carriage of MRSA, although long term use is associated with the development of resistance, and recurrence is common after short term use (17). Other control measures that have been used successfully include improved hand hygiene, contact precautions and the cohorting of healthcare workers and colonized infants.

Methicillin-susceptible Staphylococcus aureus (MSSA)

The epidemiology of and risk factors for healthcare associated infections caused by methicillin-resistant *S. aureus* (MRSA) have been comparatively well-studied; less is known about methicillin-susceptible *S. aureus* (MSSA), especially in the NICU setting. Though MSSA is generally considered an endemic pathogen, there are reports of clusters or mini-epidemics of disease among hospitalized neonates, both in well-baby nurseries and in NICUs. The primary mode of transmission in neonatal nurseries is thought to be from the hands of staff, although *S. aureus* can also be carried in the nose and/or rectum

of staff (18). Overcrowding in neonatal nurseries has been shown to be a major factor in transmission of organisms among infants. There has been little study of mixed outbreaks of MRSA and MSSA (13) and infection control strategies to control these outbreaks. Because several infection control strategies are normally instituted simultaneously in order to halt the epidemic as soon as possible, the relative importance of specific measures is unclear. In one study, the number of infants who were known to be colonized in the umbilicus with *S. aureus* at the Darlington Maternity Hospital increased from 6.4% to 8.5% between 1981 and 1987. There was no evidence of any relationship between colonization and changes in either birth rate, new intakes of clinical staff or repairs and maintenance to the old building. However, infants were normally only cultured if they were thought to have a clinical infection, so colonization rates were probably underestimated (19).

Morbidity and Mortality of Healthcare associated Staphylococcus aureus

Though healthcare associated *Staphylococcus aureus* is a well-known pathogen, the risk factors, other epidemiological data, and economic costs are still incomplete (20). Among patients in NICUs it is associated with substantial morbidity and mortality. Outbreaks caused by *Staphylococcus aureus* have been described in neonatal nurseries since the late 19th century, with large epidemics in the 1920s, 1950s, and early 1970s. The database of the Centers for Disease Control and Prevention's National Nosocomial Infections Surveillance System (NNIS) in 1996 showed that 22.2% of wound infections, 16.7% of pneumonias, and 7.5% of late-onset sepsis in NICUs were due to *S. aureus* (21). In fact

Staphylococcus aureus was identified as the most common etiological agent of healthcare associated infection between 1990 and 1996 by NNIS. Since *S. aureus* is generally a healthcare associated pathogen among neonates, it is rarely linked to early onset neonatal sepsis (~2% of cases, according to one study). However it is also a vaginal commensal, and thus potentially an early onset sepsis pathogen in the neonate.

The degree of morbidity and mortality attributable to *S. aureus* has been variably quantified and estimated in a number of studies. Noskin et al. report that a patient infected with MRSA is five times more likely to die than other patients (35). Cosgrove et al, in a meta-analysis of 31 studies, conclude that MRSA bacteremia is associated with an increased mortality compared with MSSA bacteremia with an odds ratio of 1.93 (95% CI, 1.54±2.42; P<0.01). However, only seven of the studies found a significant difference between the mortality rates of MRSA and MSSA bacteremia. In addition, Wyllie et al. report a mortality rate of 34% within 30 days among patients infected with MRSA, while among MSSA patients the mortality rate was 27%. There are a number of factors that may be immediately responsible for a patient's death, and it is believed that patients with MRSA bacteremia are sicker and consequently have a higher mortality because of their underlying illness. However, several studies that have adjusted for underlying disease still found MRSA bacteremia to have a higher attributable mortality than MSSA bacteremia (36).

Sepsis in the Neonatal Intensive Care Unit

Due to advances in the care of very low birthweight (<1500 g) and extremely low gestational age (23-28 wks) infants, resulting in longer hospital stays in neonatal intensive care units, there has been an increase in healthcare associated infections over the past two decades (while organisms contracted from the mother at birth have decreased). Most infants who become septic have been hospitalized in neonatal intensive care units for weeks or months because of extreme prematurity, congenital malformations or surgery.

Neonatal sepsis is usually classified as early or late and conclusions can be drawn about the identity of the offending organism based on the time of onset and the clinical picture. Most (~85%) of newborns with early-onset infection present within 24 hours, 5% present between 24-48 hours, and the remainder between 48 hours and 6 days of life. Though sepsis is rare in advanced tertiary care facilities (2-4 per 1000 live births), its occurrence and rate of onset increases in premature neonates (22). Causes of early-onset sepsis syndrome, associated with acquisition of microorganisms from the mother, include Group B streptococci*, Escherichia coli, Hemophilus influenzae,* and *Listeria monocytogenes.* Late-onset sepsis syndrome typically occurs at 7-90 days of life and is acquired either healthcare associatedy or from the caregiving environment. The most commonly implicated organisms in late-onset sepsis syndrome include coagulase-negative staphylococci, *Staphylococcus aureus*, *E coli* and *Klebsiella*. However during outbreaks, *Staphylococcus aureus* may be the predominant pathogen: in blood culture proven

bacterial sepsis over a six month period in an Indian nursery, 61.5% were due to *S. aureus*, 66% of which were methicillin resistant (40). The infant's skin surface, respiratory tract, conjunctiva, gastrointestinal tract, and umbilicus may become colonized from the environment, leading to the possibility of late-onset sepsis from invasive microorganisms. Vectors for such colonization may include vascular or urinary catheters, other indwelling lines, or contact from caregivers with bacterial colonization.

The most common risk factors associated with early-onset neonatal sepsis include maternal Group B streptococcal colonization (especially if untreated during labor), premature rupture of membranes, preterm rupture of membranes, prolonged rupture of membranes, prematurity, maternal urinary tract infection, and chorioamnionitis. Late onset sepsis is associated with the following risk factors: prematurity, central venous catheterization (duration of >10 days), nasal cannula, continuous positive airway pressure use, H2 blocker/proton pump inhibitor use, and gastrointestinal tract pathology.

Pneumonia is more common in early-onset sepsis, whereas meningitis and bacteremia are more common in late-onset sepsis. Premature and ill infants have an increased susceptibility to sepsis with subtle nonspecific initial presentations; therefore, they require much vigilance so that sepsis can be identified early and treated effectively. The clinical signs of neonatal sepsis are nonspecific and are associated with characteristics of the causative organism and the body's response to the infection. Complicating the process of diagnosis is the fact that these nonspecific clinical signs of early sepsis syndrome are also associated with other neonatal conditions, such as respiratory distress

syndrome (RDS), metabolic disorders, intracranial hemorrhage, or a traumatic delivery.

Given the nonspecific nature of these signs, initiating treatment for suspected sepsis is

prudent. Therefore, antimicrobial therapy and attempts to isolate a pathogen usually

proceed simultaneously, based upon clinical indications of the likely organism (22).

In the Australian nursery study spanning 30 years, infants with MRSA sepsis were

significantly smaller than infants with MSSA sepsis (mean birth weight 1090 vs. 1610 g)

and more preterm (mean gestation 27 vs. 30 weeks). Moreover, the mortality of MRSA

sepsis was 25% compared with 10% for MSSA infections. The mortality of early onset

MSSA sepsis, however, was 39% (seven of 18) compared with 7.3% for late onset MSSA

infection presenting more than two days after birth (14). This indicates that the risks of

MSSA infection are quite significant.

Strain Typing: Analysis of Chromosomal DNA by Pulsed-Field Gel Electrophoresis

Relatedness of different strains of bacteria was determined in the past by specific

serotyping, antibiotic sensitivity patterns, and more recently, on the basis of genomic

variation by using pulsed-field gel electrophoresis (PFGE). The transmission of

staphylococci that colonize the respiratory systems of infants in a nonepidemic situation

has not been well characterized by this latter method and may be the key to understanding

the usual mode of spread of organisms in the NICU. The technique, developed by

Schwarz and Cantor (23), is based on the digestion of bacterial DNA with restriction

endonucleases that recognize a few sites along the chromosome, generating large

fragments of DNA (10-800 Kb) that are not effectively separable by conventional electrophoresis. In PFGE, the orientation of the electric field across the gel is periodically changed (pulsed), allowing DNA fragments on the order of megabase pairs to be distinctly separated according to size (25). Thus, PFGE allows for the comparison of chromosomal DNA with much simpler profiles than those generated by high-frequency restriction endonucleases. All bacteria can theoretically by typed by PFGE, and the results are highly reproducible.

PFGE has been used for the investigation of MRSA/MSSA and has been compared with other methods in several studies (26-27). Even though a number of restriction endonucleases have been tested, none has shown better performance than *Sma*I. All isolates are typeable and standard strains are reproducible, even after extensive subculturing. The discriminatory power is equal to or superior to phenotypic techniques as well as to genotypic techniques such as ribotyping and PCR. PFGE has many of the characteristics attributed to an ideal typing technique and has been proposed as the gold standard for MRSA/MSSA typing. However, there are limitations for the use of PFGE, such as the long time interval until the final results are obtained (3-5 days) and the high cost of reagents and specialized equipment used for this technique. Even though the total number of bands generated is relatively small, there are problems in the interpretation of results, especially in inter-laboratory studies, as small differences in electrophoresis conditions can alter the distance traveled by each band, complicating the comparison between isolates submitted to electrophoresis in different gels. However, these

limitations do not prevent PFGE from being considered an extremely useful technique used in the characterization of outbreaks.

PFGE techniques have been extensively used to study the epidemiology of epidemic *S. aureus* strains. In these situations, the interpretation of the profiles yielded by PFGE is aided by published recommendations. Tenover et al. (28) proposed a standardized interpretation scheme in order to determine the genetic relationship between strains. Using this scheme, isolates that possess the exact same PFGE profile are considered as being identical. Isolates that differ by a single genetic event, reflected by a difference in one to three bands, are considered as being probably related. Isolates that differ in four to six bands (representing two independent genetic events) are considered as being possibly related, and isolates possessing a difference in more than six bands are considered as being unrelated. It is important to highlight that such criteria are applicable only to the analysis of a small number of isolates obtained during epidemiological studies of outbreaks in hospitals or communities during a relatively short period of time (1 to 3 months), where presumably, the genetic variability is limited. These criteria are not applicable for the study of large collections of microorganisms collected during periods of over one year (28).

II. SPECIFIC AIMS

Analysis of the natural population dynamics and expansion of pathogenic clones of *S. aureus* provides evidence that essentially any *S. aureus* genotype carried by humans can be a life-threatening human pathogen but that certain clones are more virulent than others. When the incidence of MRSA infection increased in our NICU, we sought to expand our understanding of the epidemiology of MSSA and its potential for transmission in the NICU. We used molecular typing and a retrospective cohort study to determine MSSA colonization rates and the clonal spread of strain types. The aim of this work was to characterize the MSSA strains in the Neonatal Intensive Care Unit at Yale-New Haven Hospital.

The outcomes of interest are:

1) to establish the basic epidemiological profile of MSSA by calculating incidence and prevalence of colonization, likelihood of asymptomatic carriage, and analyzing potential risk factors such as birth weight, age, length of stay, preventative measures, and use of invasive therapeutic modalities;

2) to investigate the appearance of unique versus shared strains and the pattern of spread between rooms in the NICU;

3) to analyze colonized and infected infants from date of acquisition until discharge to ascertain a longitudinal picture of colonization and to determine if culture negative status is achieved in response to antibiotic treatment.

III. PATIENTS AND METHODS

The incidence and transmission of *Staphylococcus aureus* species isolated for infection control purposes in a Level III-IV neonatal intensive care unit were investigated by molecular typing of endemic strains using pulsed-field gel electrophoresis, and in vitro examination of antibiotic susceptibilities. As a nursery specializing in the care of premature and otherwise sick newborns, the NICU is organized into 4 main rooms each serving 12 infants, and one smaller room (typically used for overflow or isolation purposes). Rooms 3 and 4 are intensive care nurseries, and rooms 1 and 2 are continuing-care nurseries for infants who do not require mechanical ventilation, arterial blood gas monitoring, or blood pressure support. The average daily census is 44 infants. Two medical teams are assigned in the NICU; each team is composed of an attending neonatologist, 3 or 4 house staff, and a medical student. One team (team A) is responsible for covering rooms 1 and 3, and the other team (team B) is responsible for rooms 2 and 4. The nursing staff may be assigned to any room, according to NICU needs.

Under the auspices of the Hospital Epidemiology surveillance program in the NICU at Yale–New Haven Hospital (YNHH), anterior nares samples were collected from all neonates within 24 hours of birth or admission and weekly thereafter.   Incident cases were defined as infants with a positive clinical or surveillance culture for *S. aureus*. Infants were considered colonized if either MSSA or MRSA was cultured from the anterior nares. Infants were considered infected if MSSA was actually isolated from either a normally sterile site or from cultures obtained on the basis of clinical suspicion.

Though neonatal sepsis may be defined both clinically and/or microbiologically, by positive blood and/or cerebrospinal fluid cultures, in this study only microbiologically proven cases were included. After approval # 27473 for medical record review was obtained from the Yale Human Investigations Committee, patient demographics and other epidemiologic data such as birth weight, age, therapeutic modalities, length of admission, presence of infection, and antibiotic use was gleaned from clinical summaries. In particular, attention was paid to therapeutic modalities such as endotracheal intubation, mechanical ventilation, umbilical catheters, central venous catheters, chest tubes, VP shunts, and feeding tubes whenever these were encountered in the chart. The actual culturing of infants and typing of strains was exempt from Human Subjects approval or formal patient consent because they were performed as part of infection control surveillance..

Alongside universal glove use in the NICU for all patient contact involving secretions, gauze pads were placed over the infants' eyes when suctioning to avoid contamination with respiratory tract secretions. Although infants were only infrequently moved from one cohort nursery to another, nursing assignments often required personnel to move between more than one nursery during a single shift. Some infants were transferred out of their cohort units because of the length of their hospitalization or due to a change in their clinical condition. Following discharge of an infant from a cohort nursery, the incubator and other environmental surfaces and equipment were thoroughly cleaned and disinfected. Environmental cultures and surveillance of the NICU staff for MSSA were not performed. Nursing personnel used individual gowns only when handling an infant

outside an incubator or bassinet. All other personnel wore short-sleeved gowns when handling infants within an incubator or bassinet. They were required to change gowns for each individual infant only if the infant was handled outside the incubator or bassinet.

Empiric antibiotic treatment was given to infants who were suspected of having sepsis, depending on their risk factors and clinical conditions. The antibiotics most commonly used empirically were ampicillin and gentamicin

Nasal specimens were collected with cotton-tipped swabs inserted into both nares and plated on both colistin-nalidixic acid (CNA) plates to verify that they were gram positive, and then mannitol salt agar (MANN) plates specific for *S. aureus.* The plates were incubated at 37°C for 24 hours. Colonies were identified as *S. aureus* by Staphaurex (Murex Biotech Limited, Kent, UK) and antibiotic susceptibilities were subsequently determined with the Kirby-Bauer procedure using oxacillin disk diffusion tests performed on Mueller-Hinton agar.

Molecular typing was performed by digesting chromosomal DNA from isolates with *Sma*I restriction endonucleases and separating the fragments using PFGE (CHEF DR-II, Bio-Rad Laboratories, Hercules, CA) performed in a 0.8% agarose gel by using the following run parameters: 198 V, 1-20 s for 15hrs. Banding patterns and strain relatedness was interpreted according to previously published guidelines (28).

Statistical Analysis

For the study period lasting 21 months, from April 2003 to December 2004, incidence and prevalence rates for *S. aureus* colonization were calculated. SAS Version 9.3 (Carey, NC) was used for statistical analysis. For describing the temporal dynamics of *S. aureus*, we computed the incidence as the number of newly detected infections on weekly surveillance divided by the total number of infants surveyed/cultured.

IV. RESULTS

During the 21 month study period, 1081 infants were screened for *S. aureus*. Of these, 877 (81.1%) tested negative, and 156 (14.4%) tested positive for MSSA. Of the infants who were culture positive for MSSA on routine surveillance, 85 charts were available for detailed time course analysis, and among them there were just 16 shared strains (gel pattern ≤ 2 bands different) accounting for 63 infants, with the remaining 22 patterns representing unique strains. Table 4 catalogs the strain types and number of infants with each strain type—those types only appearing once or twice have been grouped.

The *percentages* of total infants who tested positive for MSSA by length of stay are depicted in Figure 1. The *number* of total infants who tested positive for MSSA by length of stay are depicted in Figure 2 (MRSA trends have been added to these charts for comparison purposes). To summarize, the prevalence of colonization with MSSA approached 45% by the 5th week of hospitalization for any given infant, and 70% in 9 weeks. The age-specific prevalence of colonization with *S. aureus* had the following breakdown: from birth or admission, the prevalence of colonization rises from 4% to 15% in week 1, and then to 19%, 29% and 45% in the succeeding weeks of the hospital stay, respectively.

Following the institution of routine nasal culture surveillance and re-emphasizing the importance of infection control measures among NICU staff in April 2003, the incidence of MSSA cases dropped from 6.5 to 1.5 per 1000 patient-days per month as shown in

Figure 3. Ongoing MRSA transmission during the same period was also sharply reduced, as the incidence of MRSA cases fell from 5.8 to 0.4 per 1000 patient-days per month and a total of 48 (4.4%) infants tested positive for MRSA by the end of our study (one predominant clone – "9").

The mean length of stay among MSSA colonized patients was 73 days and the median was 57 days with an inter-quartile range between 27 and 104 days. The median length of stay was significantly longer in the intensive care infants compared to the continuing care i.e. "feed-and-grow" infants (median 77 versus 44 days, wilcoxon p=0.05). The mean length of stay was also longer in the intensive care infants, although this did not reach statistical significance (86 versus 66 days, student's t-test p=0.18). On chi square analysis, infants in intensive care rooms were also found to have a significantly higher prevalence of MSSA colonization compared to the continuing care rooms in the nursery (54% vs. 35% of the total pool, $P < 0.04$).

Pattern of transmission

The spatial and temporal distribution of MSSA strains throughout the 5 rooms of the NICU is schematically shown in Illustration 1. Molecular typing using PFGE demonstrated three more prevalent, endemic MSSA clones: clone "4", clone "15", and clone "23". Though some studies have found that MSSA isolates seldom represent the spread of clones within the hospital, in our NICU these three strains, "4", "15" and "23" spread within a one month period (though not concurrently) and accounted for 6 (7%), 9

(11%) and 10 (12%) colonized or infected infants respectively. The percentages

associated with the MSSA clones were calculated using the subset of 85 MSSA patients

whose charts were available to plot time course from birth or admission until discharge.

However the presence of strains shared between rooms demonstrated transmission across

all strain types. Corresponding to the first period of increased incidence, it was apparent

that clone "23" predominated in room 2, although its reservoir was a set of triplets that

spent most of their time in room 3. Clone "4" was mostly associated with room 1,

although its emergence, disappearance and later recurrence could not be associated with

any particular infant reservoir. Confirming the heterogeneous nature of MSSA

colonization, a general pattern emerged of strains shared and passed on within 2-3 weeks

in proximity, then disappearing as new strains arose and replaced them, and sometimes

reappearing in 1-2 infants. Since HCWs were generally not cultured the reservoir for the

reappearance of old strains cannot be confidently pinpointed. The lack of other infants

with the strain at that time of reappearance would suggest a third source such as parents

or other visitors – unless infants with the old strain were not detected somehow, but it

was in fact present. Of note, four sets of twins and one set of triplets shared the same

MSSA strain. This could be due to parents (or HCWs) who tend to care for the multiples.

Further, six infants became MSSA culture negative during the period of the study. This

could have been due to initiation of antibiotic treatment for an infection with MSSA or

other infection for which the antibiotics have activity against MSSA. Table 2 provides a

summary of antibiotic usage in the MSSA population. The majority of ampicillin/

gentamicin usage was within 48hrs of birth, with occasional longer courses. The

remaining antibiotics were utilized at various times both pre- and post-colonization with

MSSA, for sepsis, necrotizing enterocolitis, soft tissue infections, line infections, and other clinical indications such as surgical prophylaxis.

A pulsed-field gel electrophoresis of selected methicillin-susceptible *S. aureus* strains is shown in Figure 4. MSSA isolates were digested with SmaI and separated at 198 V for 15 hours with a switch time of 1–20 seconds, eventually showing 3 different patterns between lanes 1-5: isolates 1, 2 & 4, and 3 & 5, representing distinct MSSA strains.

More detailed clinical and demographic information on the MSSA patients is presented in Table 1. The mean age on admission was 5.7 days, though a majority (87.1%) were admitted at birth. There were more males than females (59% vs. 41%) and 46% received broad-spectrum antibiotics. During the study, the likelihood of developing MSSA bacteremia and/or sepsis was 4.7% (4/85) based on positive cultures.

V. DISCUSSION

The purpose of this study was to determine the incidence and characteristics of methicillin susceptible *S. aureus* colonization in newborn infants. Previous studies of pediatric patients have not compared the prevalence of nasal *S. aureus* colonization of infants in an acute illness ward with intensive support/monitoring (room 3 and 4) with that of infants who were primarily stable patients remaining in the hospital to gain more weight (room 1 and 2), nor have age-specific methicillin-susceptible *S. aureus* colonization rates been reported. The finding that infants in intensive care rooms 3 and 4 had a higher prevalence of MSSA isolates than continuing care rooms 1 and 2 was statistically significant ($P < 0.04$). Since the infants in intensive care had a significantly longer length of stay (a mean of 86, vs 66 days for continuing care infants), this result would be expected given that length of stay was positively correlated with prevalence.

In our NICU, the rate of confirmed MSSA sepsis, whether of early or late onset, was only 2.6% (4 cases out of 156 colonized patients) perhaps indicating the effectiveness of prompt identification and empiric treatment – this could also mean that colonization actually represents a low risk for ensuing sepsis, but further study would be necessary. The four known cases of sepsis were based on cultures and the judgment of the clinical team before the infant was documented as septic. Two of these infants did not survive, but it is worth mentioning that this may not reflect the true mortality of MSSA sepsis in this NICU for two reasons: 1) all-cause mortality figures were not computable due to the inability to obtain charts for some other deceased patients, whether MSSA colonized or

not, and 2) even in the charts of deceased patients that were reviewed, the documentation

of sepsis as the direct or sole cause of death was very rare. The apparent lack of certainty

in the charts over what constituted the true cause of death, especially in critically ill

children with multiple other co-morbidities, creates a difficulty in our analysis that is only

compounded by a retrospective study design. In addition, the fact that most infants

received broad spectrum antibiotics at birth (ampicillin/ gentamicin) and repeatedly at the

earliest sign of fever, nonspecific irritability, or respiratory distress due to an unknown

cause further complicates the clinical picture.

MSSA colonization of the nares, umbilical stump, and skin is a normal process.

However, staphylococcal infection occurs more frequently in otherwise healthy infants

who are colonized with *S. aureus.* Thus the relationship between colonization and

infection is not simple, but is associated with factors intrinsic to the host and to the strain

of *S. aureus,* as our study alludes to in the comparison between colonization rate among

intensive care vs. continuing care infants. However, our study did not have enough cases

of overt infection to make statistically significant conclusions between these two groups.

Even through a nested-control comparison with un-colonized infants matched for date of

admission and gender, only the length of stay and birthweight < 1500 g were significant

metrics: they are neither wholly independent factors in of themselves, or constitute a

particularly novel finding.

This study examined the epidemiology of MSSA in a level III-IV NICU in a free-

standing children's hospital located in a medical center. Although outbreaks of *S. aureus,*

particularly MRSA, are well described, there are few recent studies describing the dynamics between endemic and epidemic infections with MSSA in NICUs. In part, this may be due to increased recognition of the danger of potential outbreaks caused by multidrug-resistant organisms compared with relatively susceptible organisms. Early studies used phage typing to identify outbreak strains of *S. aureus* (31). We expanded these earlier observations using PFGE molecular typing and demonstrated periods of increased incidence of MSSA colonization or infection associated with specific clones and apparent reservoirs in infants with prolonged hospitalization (Illustration 1). Simultaneously, there were numerous diverse clones associated with infection and colonization in the NICU. Healthcare associated transmission was confirmed by the detection of sequentially predominant clones of *S. aureus*. We found periods of increased MSSA incidence, three of which were confirmed to be caused by unique clones. We hypothesize that the reappearance of clone "4" in August 2004 was likely due to a particular colonized infant with prolonged hospitalization who served as a reservoir for this clone. Similarly, we suspect that two infants with prolonged hospitalizations served as the reservoirs for clone "23" which predominated in December 2003 (Illustration 1). We did not culture the anterior nares of healthcare workers to detect staff colonization with these clones. However, healthcare workers could also have served as reservoirs for these clones as has been previously described in a pediatric cardiothoracic surgery unit where 3 children acquired surgical-site infections and 4 healthcare workers carried the strain responsible in their nares (37) -- no additional cases were identified after staff were decolonized.

Due to the extremely heterogeneous nature of MSSA colonization, and the identification

of clones definitively associated with particular colonized infants or groups of infants,

reappearance of clones was not thought to be due to contact with persistently colonized

health care workers. It must be borne in mind however, that staff included NICU

personnel, consult physicians, ancillary members such as respiratory therapists,

electrocardiogram and radiology technicians, phlebotomists, and perfusionists. Another

finding of interest was that four sets of twins and 1 set of triplets shared the MSSA strain

of their sibling(s): one could hypothesize that this may point to contact with colonized

parents and visitors as one source of strain acquisition and spread. Though only six

infants became MSSA culture negative during the period of the study, this was most

likely due to initiation of antibiotic use for unrelated conditions (pre-surgical prophylaxis,

treatment of necrotizing enterocolitis, or clinical infection due to another organism).

Previous studies in this NICU have demonstrated that 75% of newborns and >90% of

very low birth weight infants were treated with antibiotics beginning in the first 48 h of

life (29). Other NICUs report similar use of antibiotics.

In addition to PFGE analysis, our study could have benefited from using comparative

DNA sequencing of the variable number of tandem repeat regions in the *S. aureus* protein

A (spa) gene. Known as the spa typing method for genetic analysis, it is especially

effective (and enhances classification) when the investigator is blinded to the source of

the *S. aureus* isolates and the PFGE pattern, allowing an extra level of discriminatory

power to come into play that could differentiate between sub-strains < 3 bands different,

that cannot be separately classified using PFGE alone. This would in turn enhance the ability to follow the spread of these clones more specifically in our NICU.

Nonetheless, the overall percentage of colonized infants in our study was similar to the findings of an early study in 1958 but lower than those of a recent Swedish study which found that 90% of infants receiving dry cord-care were colonized with *S. aureus* in the umbilicus at the time of discharge (32). Their study showed that the risk of post-discharge disease was related to the degree of pre-discharge staphylococcal skin colonization. Though *S. aureus* initially colonizes the umbilical stump and readily spreads to other sites in neonates, nasal colonization may be more persistent than that of the umbilicus. This may help to explain the inconsistent findings of the various studies. Regardless, our observations of a high colonization rate signal the need for similar studies in other NICUs to see if there are comparable colonization rates, as well as repeat studies in our own NICU to determine if this rate is normal given the current standard of health care hygiene. Regardless, high colonization rates coupled with the finding of length of stay as a risk factor, underscore the continuing need to try to decrease length of stay in NICUs. Length of stay has been reported as a risk factor in both univariate and multivariate analyses for acquisition of healthcare associated colonization and infections (33). Infants with longer stays presumably had more healthcare worker contacts and opportunities for transmission of strains from other infants. In our study, the mean length of stay among MSSA colonized patients was 73 days, and the median was 57 days with an inter-quartile range between 27 and 104 days. We suspect that infants in the continuing care nurseries required only short-term intravenous access and were relatively

well, having fewer contacts with healthcare workers, and therefore had fewer opportunities to become colonized with an epidemic clone. However, commonly used interventions that imply increased contact between staff members and patients, such as respiratory support by nasal cannula, were not associated with a higher risk of colonization. Male gender was also not a significant risk factor for MSSA colonization in the final analysis.

VI. CONCLUSION

The monitoring of staphylococcal disease in nurseries can be a valuable surveillance technique in predicting outbreaks. This is important when one considers that the majority of infection control budgets are very limited and hospital epidemiologists must apportion the resources judiciously. Control of MSSA tends to be challenging because colonization is expected, endemic infections are considered expected and surveillance usually focuses on drug-resistant pathogens as treatment options are limited. During the period of study for this critically ill infant population, the incidence of MSSA (and MRSA) colonization fell dramatically in response to stringent infection control practice. We also identified an increased risk for MSSA colonization among intensive care infants as compared to continuing care infants.

An important finding in this study was the pattern of spread of strains of *S. aureus* among infants between and in the same NICU room. The finding of specific strains associated with certain rooms makes it unlikely that there was a common source of contamination in the common areas. Some strains appeared to spread in a stepwise pattern, in which a new infant or infants each week became colonized with the same strain that previously colonized an infant who was discharged when surveillance cultures of new infants were obtained. Some strains were present almost throughout the entire study period and demonstrated near continuous transmission from infant to infant, whereas others were present for only a short period of time. This suggests that some infants who reside in the

NICU for extended periods may act as reservoirs for *S. aureus* and transmit them to infants they come in contact or through shared care-givers.

The exact mode of transmission of *S. aureus* from infant to infant was not proven. However, evidence suggests that hand carriage, as NICU staff may neglect to perform hand hygiene appropriately before and after every infant contact, may be responsible. The relationship among colonization, infection, and transmission is a dynamic process and no doubt is influenced by numerous factors, including host factors, intrinsic microbial factors, and infection control practices. Since most experts do not recommend routine surveillance, surveillance should be directed to investigating outbreaks of healthcare associated infections rather than colonization whenever possible.

These findings emphasize the need for cost-effective surveillance strategies for endemic infections in order to monitor the progression from colonization. Finally, knowledge of the pattern of healthcare associated infection can contribute to the intensification of infection control measures and the updating of antibiotic usage guidelines.
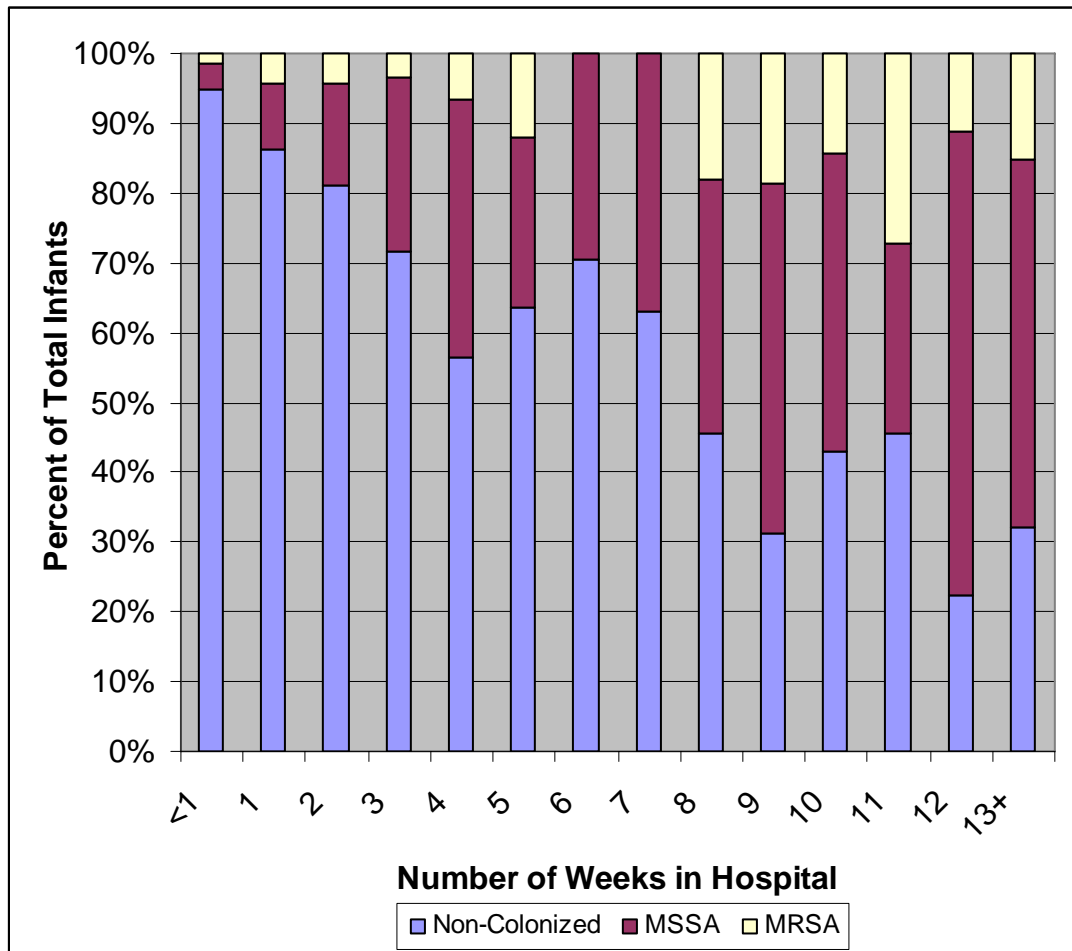
VII. FIGURES

Figure 1. Percent of infants colonized with MSSA or MRSA by length-of-stay.

The total number of infants during the time period was 1081. Prevalence of colonization

from birth to discharge, at 1-week intervals (the percentage of cultures with S. aureus

increased almost linearly with infant age). For the purpose of data analysis, only the

first isolate of each organism (MSSA or MRSA) was included in the calculation.

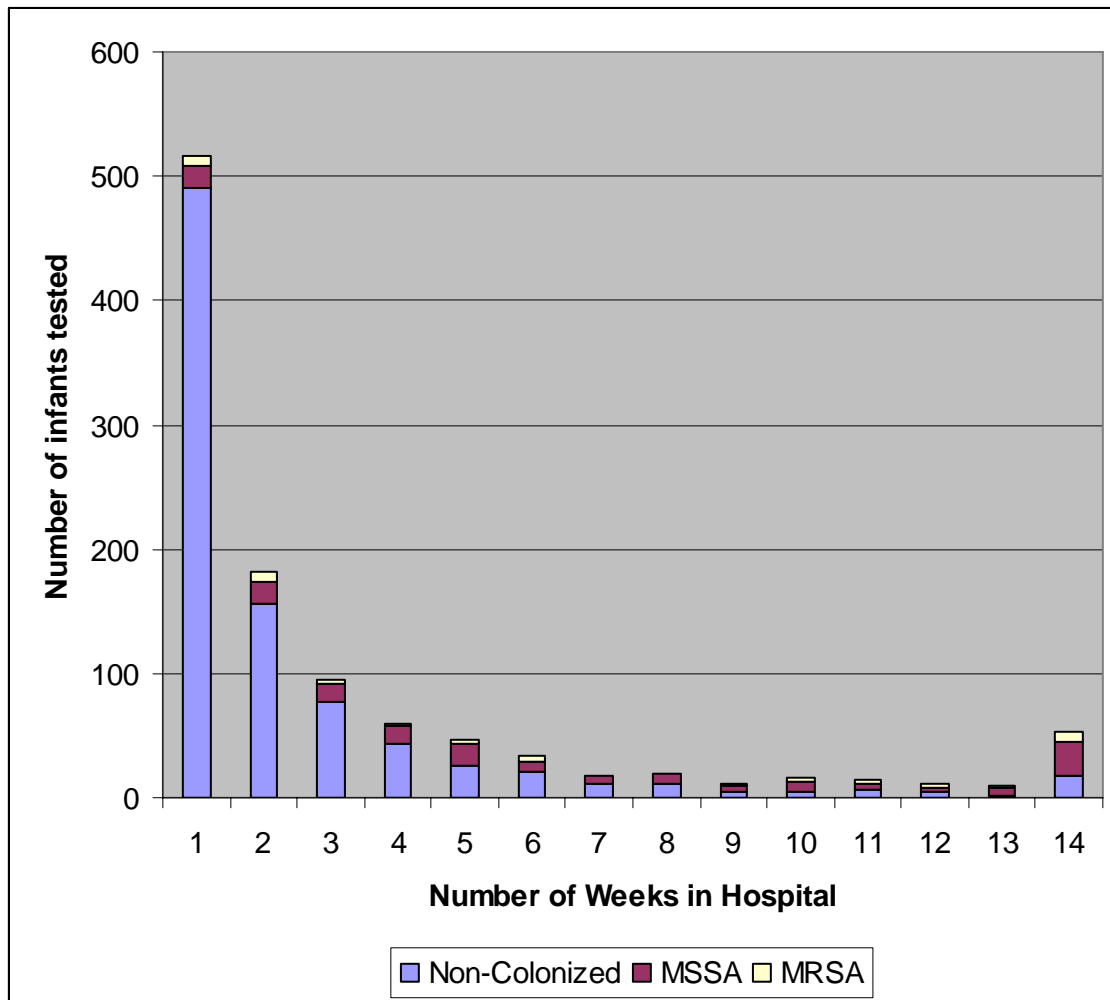**Figure 2.   Number of infants colonized with MSSA or MRSA by length-of-stay.**

The total number of infants during the time period was 1081.
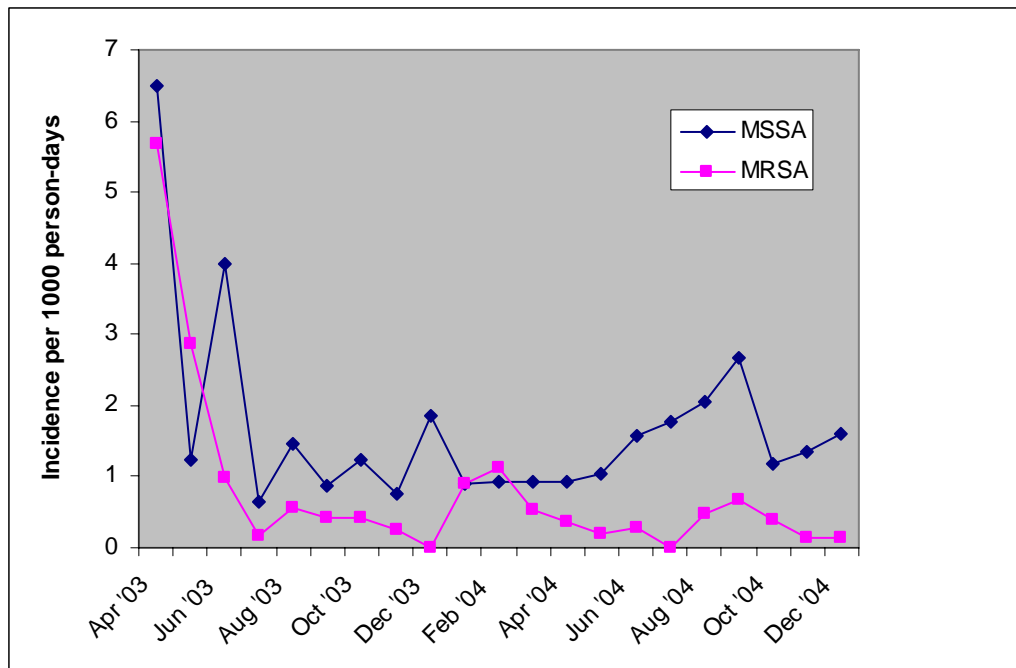
Figure 3. **Temporal Dynamics of MSSA and MRSA colonization in the NICU.**

This figure shows the incidence of cases over time since the institution of routine nasal

surveillance in April 2003. The total number of infants during the time period was 1081.

Figure 4.   Pulsed-field gel of selected methicillin-susceptible *S. aureus* strains.

MSSA isolates were digested with SmaI and separated at 198 V for 15 hours with a

switch time of 1–20 seconds, eventually showing 3 different patterns between lanes 1-5:

isolates 1, 2 & 4, and 3 & 5, representing distinct MSSA clones 7, 15, and 23 respectively.

VIII. TABLES

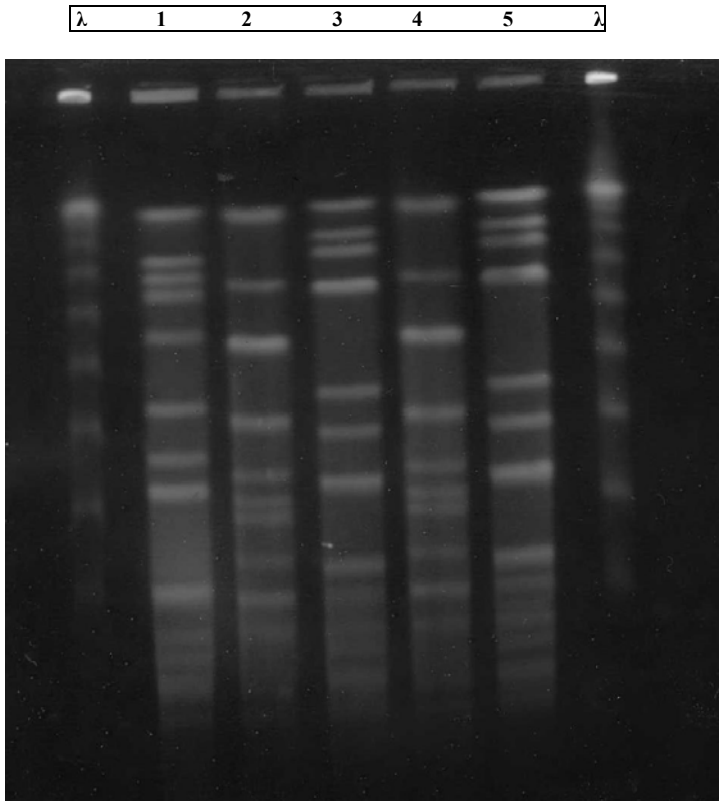**Table 1. Characteristics of MSSA-Colonized Patients (n=85)**

Characteristic	Value
Age on Admission, mean days (SD)	5.7 (19.0)
Birthweight, mean grams (SD)	1450.8 (894.0)
Birthweight, median grams (IQR)	1185.0 (860 to 1720)
Length of stay, mean days (SD)	72.9 (64.5)
Length of stay, median days (IQR)	57.0 (27 to 104)
Admitted at Birth, %	
Neonate	74 (87.1%)
Post-birth	11 (12.9%)
Gender, %	
Female	35 (41.2%)
Male	50 (58.8%)
Received Broad-Spectrum Antibiotics	
Yes	39 (45.9%)
No	46 (54.1%)
Mode of Delivery	
Vaginal	62 (72.9%)
Cesarean	23 (27.1%)
Intubated	
Yes	14 (16.5%)
No	71 (83.5%)
Received TPN	
Yes	43 (50.6%)
No	42 (49.4%)
Developed Sepsis	
Yes	4 (4.7%)
No	81 (95.3%)
Product of multiple-gestation birth	
Yes	14 (16.5%)
No	71 (83.5%)
Developed Respiratory Distress Syndrome	
Yes	23 (27.1%)
No	62 (72.9%)

Table 2.   Antibiotic usage in MSSA colonized patients (n=85)

Courses varied from 2– 7 days in most cases.

Antibiotic	# of patients
Gentamicin	25
Ampicillin	21
Vancomycin	9
Clindamycin	7
Nafcillin	6
Cefotaxime	5
Amoxicillin	3
Erythromycin	1
Cefuroxime	1

Table 3.   Intravascular line use by type of access (n=85)

Type	# of patients
TPN	43
Central venous catheters	23
Peripheral venous catheters	76

Table 4.   Strain Typing (n=85)

Strain	# of patients
23	10
15	9
4	6
1	4
26	4
24	3
33	3
2,3,9,17,18,19,27,30,31	2
5,6,7,10,11,12,13,14,16,20 ,21,22,25,28,29,32,34-39	1

IX. ILLUSTRATIONS

2 3	9	
4 4		
5		
1 3		
10 13		
14	1 2	
6		

Period 1 (Apr – Jul '03)

11 15	4 9	
19	16 17	
	20	
12 17		
18 21		
24	4 24	
15 19		
23 23		
23 24		
25 30		

Period 2 (Aug – Nov '03)

26 27	15 15	
29	27 30	
	31	
14 15		
26 27		
27 28	14	
31		
29 31		

Period 3 (Dec '03 – Mar '04)

1 15	15 23	
23 24	23	
33 34		
16 23		
23 23		
32 32		
36		
23 26		

Period 4 (Apr -Jul '04)

4 31	15 15	
36 38	37	
	4 32	
	35	
27 33		

Period 5 (Aug – Nov '04)

Illustration 1. Spatial and Temporal Dynamics of MSSA spread in the NICU.

Periods are broken into 120-day intervals.   Each box represents a different NICU room.

Numbers within each room represent patients (n=85) colonized by distinct MSSA strains

differentiated using PFGE.   Rooms are numbered 1-5 counter-clockwise from the upper

left-most box.

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