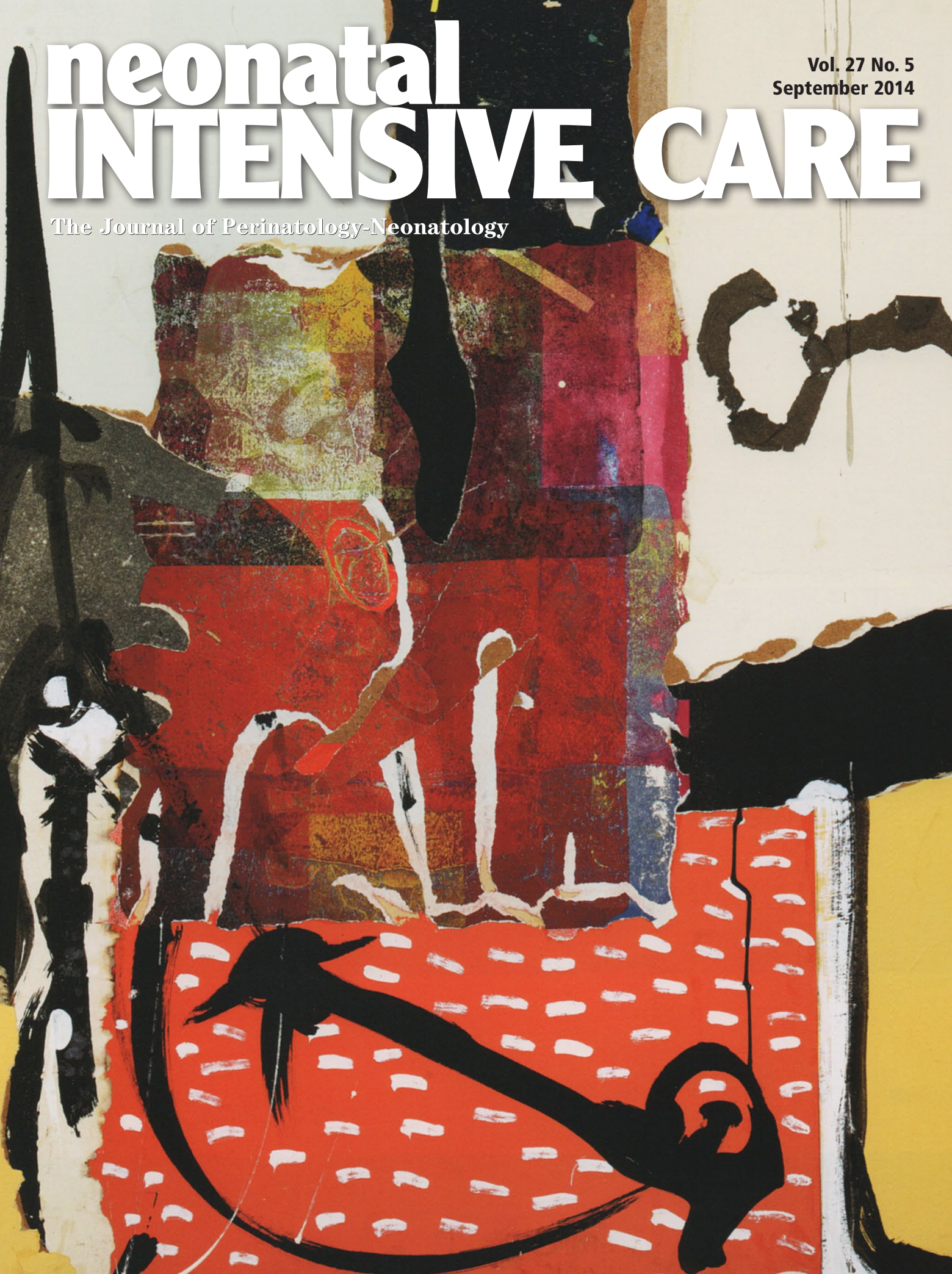
Vol. 27 No. 5
September 2014

# neonatal INTENSIVE CARE

The Journal of Perinatology-Neonatology



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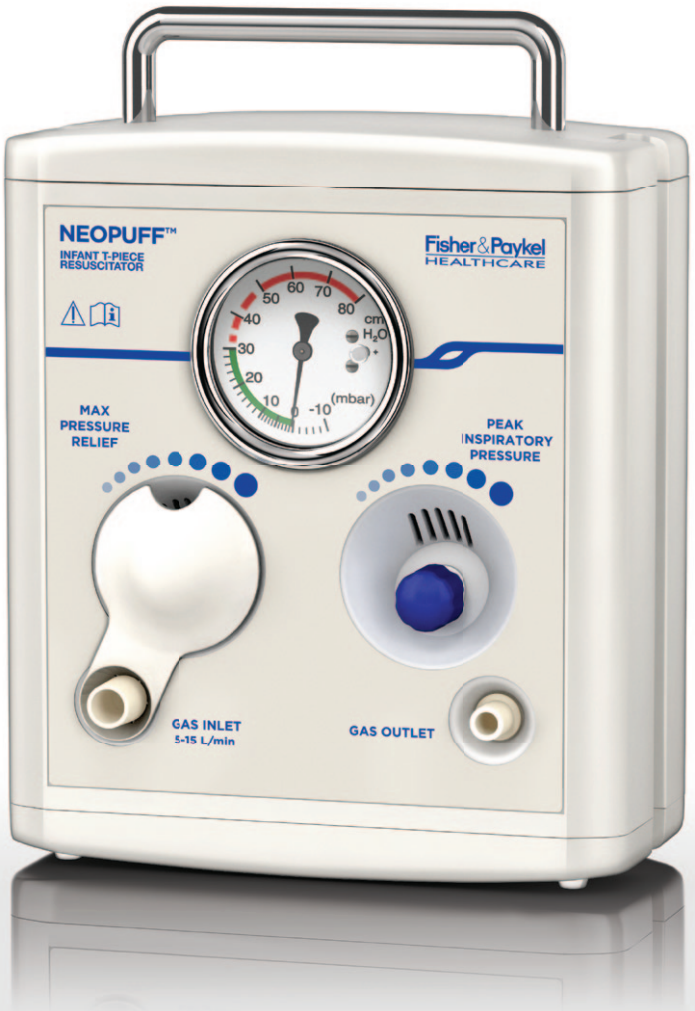


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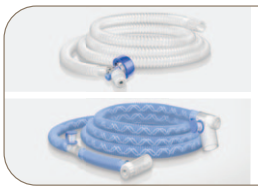

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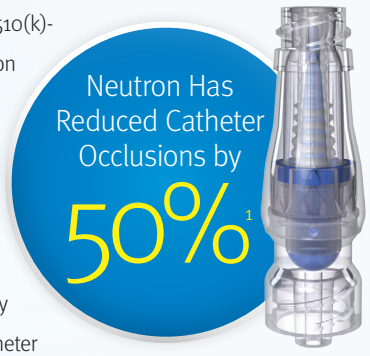


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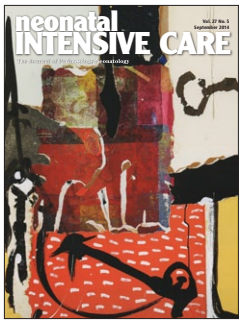


neonatal INTENSIVE CARE

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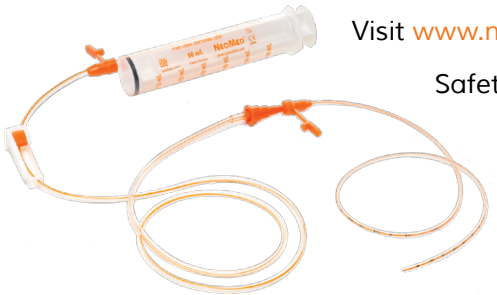

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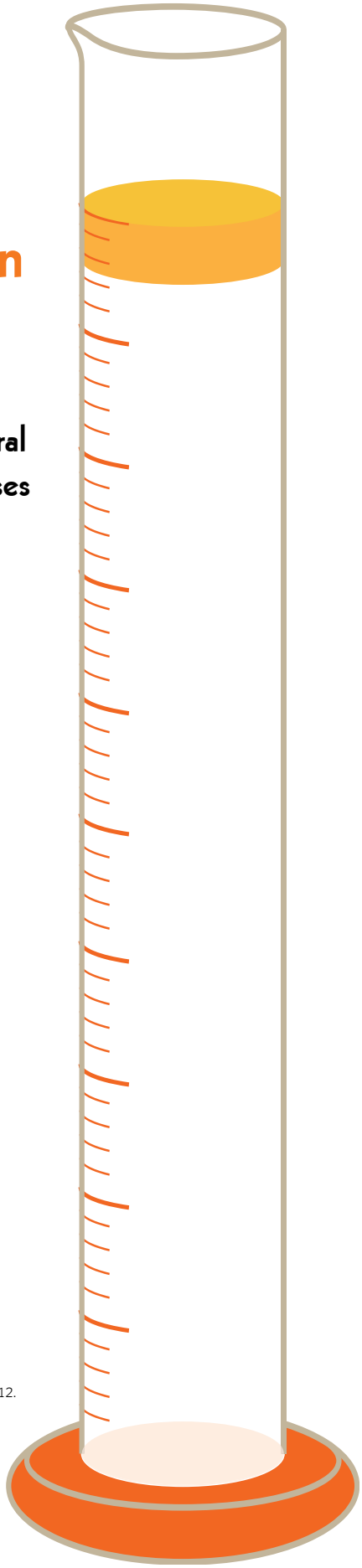

[1]Neu J, Polin R. Gastroenterology and Nutrition: Neonatology Questions and Controversies. Philadelphia, PA: Elsevier Saunders; 2012.
[2]Jensen RG. Handbook of Milk Composition. San Diego, CA: Academic Press; 1995.
[3]How NICU Syringe Choice Can Reduce Fat Loss in Human Breast Milk. NeoMed. 2014.

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1. Centers for Disease Control and Prevention. (2003) Guidelines for Environmental Infection Control in Health-Care Facilities. Recommendations of CDC and Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR, 52(RR10):1-42.

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# Can Umbilical Cord Tissue Biopsy Be An Alternative Source Of Stem Cells?

Boris M Petrikovsky, Alexander Fuks, Lali Sichinava, Evgeniy Zharov


## Abstract

**Introduction:** The purpose of this report is to introduce a new procedure—the umbilical cord punch biopsy—for recruiting Wharton's Jelly stem cells at various gestational ages.

**Material and Methods:** 50 patients were enrolled in the study. 25 had full term babies and 25 delivered between 25 and 36 weeks. After birth, an umbilical cord biopsy was performed using biopsy forceps. Collected material was placed into Earle's balanced salt solution. Cell harvesting was performed through a tissue digestion using fluorescence—activated cell sorting. We assessed a sample size amount and time of bleeding from the umbilical cord after successful biopsy.

**Results:** Successful sampling was performed on all 25 full term umbilical cords. The punch size varied from 1.3 to 3.5 square mm of cord tissue. 9 out of 25 full term umbilical cords required a second attempt. Bleeding time varied from 5 to 45 sec. In premature umbilical cords, successful biopsies were obtained in 17 out of 25 cases, all after 28 weeks of pregnancy. Bleeding time in preterm umbilical cords after the biopsy varied from 7-50. Bleeding was self-limited in all cases.

**Conclusions:** Our data demonstrated that the punch biopsy of a pulsating umbilical cord after the birth of a child can be successfully performed in most cases with minimal or no damage to the umbilical cord. We conclude that with appropriate instrumentation, experience after further studies, umbilical biopsy may become a useful procedure for the recruitment of stem cells.


## Introduction

Wharton's Jelly is a gelatinous substance found within the umbilical cord as a mixture of water, gelatin, lipids, proteins, and enzymes. Wharton's Jelly is a rich source of stem cells, fetal specific proteins, fatty acids, and phospholipids, among other components. It provides protection to the blood vessels in the umbilical cord. It is named after an English physician and anatomist Thomas Wharton who first described it in his publication Adenographia or "The Description of the Glands of the Entire Body", first published in 1656. Wharton's Jelly is composed of an acid mucopolysaccharides (35%), gelatin (25%), hyalaronan (15%), fetal-specific proteins and enzymes (gelatinase A-metalloproteinase and gelatinase B). It also contains a small amount phospholipids and glycolipids.

The authors are with Wyckoff Heights Medical Center, Queens Hospital Center, Moscow University School of Medicine or Russian Institute of Regenerative Medicine.

Wises et al [1] and Wang et al [2], were two of the first to show the potential of Wharton's Jelly as a valuable source of mesenchymal stem cells.

Wharton's Jelly multipotent stem cells have several advantages over bone marrow stem cells including non-invasiveness, the ease of procurement, reduced risk of transmissible infections and the availability for immediate use [3].

The purpose of this report is to introduce a new procedure—the umbilical cord punch biopsy—for recruiting Wharton's Jelly stem cells at various gestational ages.

## Material and Methods

50 patients who delivered vaginally or by a cesarean section were enrolled in the study. 25 had full term (36-41 weeks) babies and 25 delivered prematurely between 25 and 36 weeks.

Exclusion criteria included cord blood donors, patients with chorioamnionitis, and known other infections. After delivery of the baby, an umbilical cord biopsy was performed using punch biopsy forceps. Collected material was placed into Earle's balanced salt solution. Cell harvesting was performed through a tissue digestion using fluorescence—activated cell sorting. Since the purpose of this report is to describe the validity of umbilical cord biopsy for stem cell recruitment, details of the stem cell isolation and culture process can be found in the protocols developed by Can et al. [4]

We assessed sample size amount and time of bleeding from the umbilical cord after successful biopsy. The number of punch biopsy trials was limited to two.

## Results

Successful sampling was preformed on all 25 full term umbilical cords. The punch size varied from 1.3 to 3.5 square mm of cord tissue. 9 out of 25 full term umbilical cords required a second attempt to obtain measurable amount of tissue. Bleeding time varied from 5 to 45 seconds; bleeding was self limited in all cases. Vascular injury to the umbilical cord was detected in three cases.

In premature umbilical cords, successful biopsies were obtained in 17 out of 25 cases, all of them after 28 weeks of pregnancy.

Bleeding time in preterm umbilical cords after the punch biopsy varied from 7-50 seconds. Bleeding was self-limited in all cases.

## Discussion

A Russian research team pioneered the search for alternative sources of mesenchymal stem cells and concentrated their efforts on the umbilical cord [5]. They determined that the contents of the umbilical have a high number of mesenchymal stem cells that may be successfully expanded in culture. This is a particularly important finding because it has become increasingly clear that bone marrow may no longer represent the only suitable source of stem cells [3]. Besides the high degree of invasiveness and pain inherent in bone marrow extractions, mesenchymal stem cells are present at a very low frequency in bone marrow aspirates and this frequency declines with a donor's age [6]. Umbilical cord stem cells, on the other hand, maintain their self-renewal capacity for a longer time, exhibit shorter doubling times, and a broader pluripotency as compared to those extracted from bone marrow [7].

The morphology, immunophenotype, and differentiation potential of Wharton's Jelly stromal stem cells fully fulfill the criteria for mesenchymal stem cells proposed by the International Society for Cellular Therapy [8]. Umbilical cord stem cells possess a fibroblast-like morphology, high proliferative rate, and multipotent differentiation capacity [3,9]. These cells have a colony forming unit frequency of 1-333 [10]. The immunomodularity of Wharton's Jelly stem cells is apparent in both T and B lymphocytes.

Che et al [11], reported that mesenchymal stem cells also inhibit B cell proliferation and antibody production. In spite of their numerous benefits, Wharton's Jelly stem cells have one major disadvantage: the low total number that can be harvested from a single unit [12]. Therefore, the transportation of Wharton's Jelly stem cells is significantly more successful in neonates and young children who require a smaller quantity of cells [13]. Because the umbilical cord possesses more stem cells at younger gestational ages, the idea of the Wharton's Jelly biopsy appears attractive if proven to be safe [14,15]. Our data demonstrated that the punch biopsy of a pulsating umbilical cord after the birth of a child can be successfully performed in most cases with minimal or no damage to the umbilical cord. We conclude that with appropriate instrumentation, experience after further studies, umbilical biopsy may become a useful procedure for the recruitment of stem cells.

## References


1 Weiss ML, Mitchell KL, Hix JE.Transplantation of porcupine umbilical matrix cells into the rat brain. Exp Neurol 2003, 182; 288-299.

2 Wang HS, Hung CS, Pang ST. Mesenchymal stem cells in the Wharton's Jelly of the human umbilical cord. Stem Cells 2004, 22; 1330-1337.

3 Batsali A, Kastrinaki M, Papadaki H, Pontikoglou C. Mesenchymal stem cells derived from Wharton's Jelly of the umbilical cord; biological properties and emerging clinical applications. Current Stem Cell Research and Therapy, 2013, 8; 144-55.

4 Can A, Karahuseyinoglu S. Concise review; Human umbilical cord stroma with regard to the source of fetus derived stem cells. Stem Cells 2007; 25, 2886-95.

5 Romanov Y, Svinskaya V, Smirnov V, Searching for alternative sources of post natal human mesenchymal stem cells. Stem Cells, 2003; 21, 105-10.

6 Mueller S M, Glowacki J. Age related decline in the osteogenic potential of human bone marrow cells cultured in 3 dimensional collagen sponges. J Cell Biochem, 2001; 82, 583-90.

7 Troyer D L, Weiss M L, Concise review, Wharton's Jelly derived cells are a primitive stem cell population. Stem Cells 2008; 26, 591-9.

8 Dominici M, Leblanc K, Mueller I Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy Position Statement. Cytotherapy 2006; 8, 315-7.

9 Petrikovsky B M Wharton's Jelly peptides and skin aging. Neonatal Intensive Care, 2013; 26, 25-9.

10 Sarugaser R, Lickorich D, Bakch D, Hosseini M M, Davis J E. Human umbilical cord perivascular cells a source of mesenchymal progenitors. Stem Cells 2005; 23, 220-9.

11 Che N, Ly X, Zhou S. Umbilical cord mesenchymal stem cells suppress B-cell proliferation and differentiation. Cell Immunol 2012; 274, 46-53.

12 Migliaccio A R, Adamson J W, Stevens C E, Dobrila N L, Carrier C M, Rubinstein P, Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. Blood 2000; 96, 2717-22.

13 Gluckman F, Rocha V, Arcese W. Factors associated with outcomes of unrelated cord blood transplant; guidelines for donor choice. Exp Hematol 2004; 32, 397-407.

14 Petrikovsky B M, Karsdon J. Stem Cells – From Jelly to gold. Editorial. Neonatal Intensive Care 2011; 24, 1-25.

15 Petrikovsky B M, Wharton's Jelly protein as a stimulant of skin stem cells. Proceedings of Tenth Anti-aging Medicine World Congress, Monaco 2012; 29, 151-4.

# Bless The Technology

B M Petrikovsky, MD PhD

I recently met a very well-respected, South African-educated internist who also happens to be a close friend and colleague. He walked into the examination room carrying a hand-held laptop, which has been standard issue for most medical professionals in his line of work. Throughout the brief consultation with the patient he was largely preoccupied with maintaining the electronic medical records. He confessed to me that it was quite difficult to transition from the classical practice of medicine to computer-assisted medical examination. However, he continued to confide in me that incentives from insurance companies made the uncomfortable transition a mandatory one. Furthermore, since the passage of the Patient Protection and Affordable Care Act (Obamacare), electronic medicine has been forced upon practitioners to an even further degree.

I clearly recall my embarrassment when during my tenure at New York Downtown Hospital, part of the Cornell Presbyterian Health System, I was treating an 85-year-old great grandmother who came to me with post-menopausal bleeding. The computer program, which I was obligated to use, forced me to go through mandatory questions which included asking about the patients' sexual preferences and the time when she first saw the appearance of pubic hair. I must confess, I lied. I lacked the necessary mechanistic constitution to ask an 85-year-old holocaust survivor, mother of nine, and grandmother of 21, if she was a lesbian, practicing safe sex, and is aware of sexually transmitted diseases. I also did this for questions regarding the use of illicit drugs, smoking, birth control, etc. The computer program, which was purchased by that institution, would not allow me to continue the exam, and get to the relevant sections unless I addressed these dilatory matters. The computer system was also linked to a billing department. Namely, the more questions I ask, and the more systems I go through, no matter how irrelevant, I can bill more. There are two possible conclusions that can be drawn from these facts, that either the designers of these systems do not trust doctors as much as they trust the IT professionals, with respect to the treatment of patients, or these news systems represent a higher stream of revenue than their classical alternative.

I don't want this commentary to be an indictment of technology in medicine. Computers today are an indispensable tool in the practice of medicine, from administrative tasks such as managing the patient and doctor scheduling, to creating an easily accessible patient history, and in helping medical professionals access and analyze patient information.

Thank goodness for computers, my private practice is flourishing, and attracting patients who are sick and tired of seeing the back of the doctor, who is going through computer questions, to qualify for the appropriate level of service, which comes with a matching paycheck. If this is how we train the next generation of doctors, there will be no room for: experience, intuition, and compassion.

We can see this happening already in the world. WebMD, an online provider of medical information is a wholly accurate source of information. I say accurate because the information is factually correct, but in the hands of an untrained person the information changes from a helpful resource into a destructive force. Doctors spend, at a bare minimum, 10 years of their life, and often much longer, studying and preparing to be able to treat people who need their help. They have spent this time learning not only facts, but they have also learned to develop a medical intuition, which means that they are able to synthesize and narrow facts in order to arrive at a diagnosis. I fear a world that turns doctors into instruments of computers instead of the other way around. Sadly that is the world we live in today, and all I can hope for is that we realize the error in our ways before it is too late.

Dr Petrikovsky is the chief of maternal and fetal medicine at Wyckoff Heights Medical Center and Editorial Board Member of Neonatal Intensive Care.

# News

☐ September 2014

### Dispensers Aim to Prevent Mix-ups

NeoMed, a developer and manufacturer of neonatal enteral systems and oral dispensers, has launched its ISMP-compliant Oral Dispensers. The Institute for Safe Medication Practice (ISMP) published their 2014-15 Targeted Medication Safety Best Practices for Hospitals in January 2014. The ISMP recommends in Best Practice #5 to "use liquid medication dosing devices (oral syringes/cups/droppers) that only display volume using the metric scale (mL)". The ISMP hopes to reduce the risk of medication measurement mix-ups by standardizing the method for liquid measurement. In response to this best practice recommendation, NeoMed has removed all teaspoon gradient markings from their Pharmacy Oral Dispenser line. NeoMed offers a full line of amber and clear oral dispensers from sizes 0.5 mL through 60 mL that comply with the ISMP recommendations. NeoMed's oral dispensers feature an O-ring plunger design for smooth and accurate delivery, which aligns with distinct gradient markings for precise measurements. The plugged, hands-free, self-righting tip caps create an airtight and watertight seal to secure the dispenser's contents.

### Neptune Device Offers Adjustability

Teleflex has introduced the new Hudson RCI Neptune Heated Humidifier, which, in response to clinician feedback, includes ConchaSmar Technology features such as an Auto-Set mode, low water notification and expanded temperature range. All new Neptune Heated Humidifiers sent to a medical facility will have the following features and benefits. Neptune Heater senses a low-water condition, has a flashing visual indication that activates, and 10 minutes later, an audible alarm will activate. Also included is a choice of adjustable modes and settings. Customize treatment and absolute humidity delivered based on: patient type, ventilation mode and therapeutic goals. Auto-Set Mode allows clinicians to pre-set the temperature and gradient of the Neptune Heated Humidifier while still allowing for invasive and non-invasive toggling. There are also multiple mounting points and increased temperature rangers at the Circuit Y-piece.

### B&B Adds to Team

B&B Medical Technologies announced that respiratory sales and marketing management veteran Stu Novitz has joined its team as the company expands its growing international presence and strengthens its distributor network. Novitz brings with him a wealth of experience working with distributors within the hospital homecare and long-term care market, having recently spent the last four years at Clement Clarke spearheading the company's efforts to extend its reach into the North American medical markets. Joining in advance of B&B Medical's new product rollout, Novitz will make an immediate contribution to the company's continuing success. Novitz joins B&B Medical Technologies as Vice President of Sales and Marketing to advance B&B's reputation for providing specialty airway solutions to clinicians worldwide. "I look forward to working with the management team to further define our action plans that will ensure B&B's long-term growth strategy. I am fortunate to be joining such a respected company with enthusiastic goals for developing innovative, new products," said Novitz. B&B's newest product on the horizon is a solution for the Neonatal Critical Care market and that is only the beginning—the company's development plan is to launch new products each year.

### Breech Baby, Breech Baby, Give Me Your Hand

More women in Germany are asking to have their breech babies delivered vaginally and doctors increasingly are willing to let them try—a trend unlikely to happen in the US. Many doctors in Germany say breech birth is an appropriate choice as long as strict criteria are met and the delivery is overseen by an

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experienced physician. The approach reflects a tendency among German medical professionals to favor noninvasive remedies as a first line of attack against medical problems, doctors say. Around 8.4% of breech babies in Germany were delivered through the birth canal in 2012, up from 7.2% in 2009, according to the most recent annual German birth survey compiled by the Aqua-Institute. Vaginal breech births overall are more dangerous for babies than caesarean sections. Studies in recent years, however, have shown that a vaginal delivery can be safe under certain circumstances, including how the baby is positioned in the womb. Most doctors and hospitals in the US won't perform vaginal breech deliveries outside of emergency situations. Still, the American Congress of Obstetricians and Gynecologists softened its stance on vaginal breech birth in 2006 and reaffirmed the position in 2012. The group said it may be a "reasonable" option for women depending on the experience of the physician. It cited studies that showed breech birth under certain protocols is no riskier than a planned C-section. Most babies are positioned head down in the womb by the time they approach their due date. But breech babies are situated so that their feet or buttocks will come out first during childbirth. There are risks to vaginal delivery: If the baby's head gets stuck in the birth canal, it can cause brain damage or even death. About 4% of babies are breech at the end of pregnancy. Before trying a vaginal breech birth, many doctors will attempt to turn the baby by applying pressure to the mother's belly in a procedure called an external cephalic version. Medical professionals say breech birth isn't poised for a comeback in the US Delivering a breech baby vaginally often requires special techniques with which many doctors today aren't experienced. Fear of malpractice lawsuits also deters many physicians from attempting such deliveries. Martin Gimovsky, an attending perinatologist at Newark Beth Israel Medical Center in Newark, N.J., and a clinical professor at the Icahn School of Medicine at Mount Sinai Hospital in New York, said he began declining requests for breech deliveries about a decade ago due to their "negative stereotype" among medical malpractice insurers and the broader population. Still, he believes vaginal breech birth should be revived. "I have seen mothers die of a C-section in my own hospital," he said. Information in this article first appeared in the Wall Street Journal.

### Born Best Friends

A pair of US twin sisters who were born holding hands were breathing on their own after being removed from a ventilator, their mother has said. Jillian and Jenna Thistlethwaite shared an amniotic sac and placenta, a rare condition known as monoamniotic birth. "They're already best friends," said their mother, Sarah Thistlethwaite. They were born on Friday in the US state of Ohio, grasping each other's hands when doctors lifted them up for their parents to see after delivery. Monoamniotic birth occurs in only one in 10,000 pregnancies. Thistlethwaite, 32, was monitored for weeks at Akron General Medical Center in Akron, as monoamniotic twins are at risk from becoming entangled in each other's umbilical cords. She told the Akron Beacon Journal newspaper that holding her children was "the best Mother's Day present ever". "I can't believe they were holding hands," she said. "That's amazing."

### Jenny McCarthy Will Not Like This

A study has results that continue to debunk the dubious link between vaccines and autism, saying that boys who develop the condition are exposed to higher levels of steroid hormones in the womb than those who don't develop it. Scientists at the University of Cambridge in England and the Statens Serum Institute in Copenhagen said in a report released that prenatal levels of substances such as testosterone, progesterone and cortisol were greater on average in boys who were later diagnosed with autism. The finding, published in the journal Molecular Psychiatry supplies a possible explanation for how autism develops during pregnancy, countering fears that external factors such as vaccines play a role. About one child in 160 is affected by autism spectrum disorders, a group of brain development disorders, according to the World Health Organization. The study drew on 19,500 amniotic-fluid samples stored in a Danish biobank from individuals born between 1993 and 1999. The researchers identified samples from mothers who gave birth to 128 boys later diagnosed with an autism-spectrum condition. Because some of the hormones are produced in much higher quantities in males than in females, the finding may help explain why autism affects more boys, the researchers said. Mothers shouldn't rush to use steroid-hormone blockers, as this may have unwanted side effects, according to the researchers. The study also shouldn't be interpreted as indicating a need to develop a prenatal screening test as the results were found at the average group level and may not predict diagnosis for an individual, they said.

### Medela Adds to Family

Brea-based healthcare device maker Acacia Inc. has agreed to sell its neonatal feeding unit to Medela Inc., a global healthcare company that specializes in breastfeeding devices and medical suction equipment. Terms of the deal were undisclosed. Medela, which has its U. headquarters in McHenry, Ill., operates through 13 subsidiaries around the world, including units in France, Japan and Switzerland, where it was founded in 1961. The acquisition of Acacia's neonatal products segment is expected to help Medela expand its neonatal intensive care unit services, which are aimed at providing human milk to babies at risk. Acacia Chief Executive Dan Hyun will join Medela as a vice president. Acacia has an estimated $12 million in revenues and 25 employees. Medela has about 600 employees.

### Got Human Milk? Good

Prolacta Bioscience, the pioneer in human milk-based nutritional products, has announced that the journal Breastfeeding Medicine has published an analysis concluding that an exclusive human milk diet results in lower mortality for extremely premature infants. The peer-reviewed analysis by Steven A. Abrams, MD, Medical Director of the Neonatal Nutrition Program at Baylor College of Medicine, Richard Schanler, MD, Director of Neonatal Services at Cohen Children's Medical Center of New York and North Shore Long Island Jewish Health System and their colleagues also found an increase in the likelihood of developing necrotizing enterocolitis (NEC), NEC requiring surgery, or sepsis, as the volume of milk containing cow milk-based protein fed to the infants in the control group increased. The article, "Greater Mortality and Morbidity in Extremely Preterm Infants Fed a Diet Containing Cow Milk Protein Products," combined the data from two previously reported trials. The article evaluated extremely premature infants in the neonatal intensive care unit (NICU) weighing less than 1,250 g at birth, who received a diet consisting of either human milk fortified with a human milk protein-based fortifier, or a diet containing varying amounts of cow milk-based protein. The analysis showed decreased mortality and NEC rates for those premature infants on the exclusive human milk diet. Additionally, for every 10% increase in the volume of milk containing cow milk-based protein fed, the risk of sepsis

increased by 17.9%. Sepsis is a potentially fatal bacterial infection of the bloodstream resulting in widespread inflammation.
The analysis has been published on the heels of the Pediatric Academic Societies (PAS) Annual Meeting, which had a record number of abstracts focused on human milk-based diets for premature infants this year. Additionally, Prolacta sponsored a CME accredited symposium in Vancouver on May 4 during PAS entitled 100% Human Milk Diets: Meeting the Challenge of Infant Nutrition in the NICU that was standing room only.

### Students Build Own Ventilator

Mechanical neonatal ventilators can cost tens of thousands of dollars each, a price that's out of reach for many impoverished areas of the world. Premature babies and those born with breathing conditions often desperately need these lifesaving devices to get through the most difficult early weeks. To improve access to neonatal ventilation, students at Brigham Young University developed a basic, stripped down ventilator that can be manufactured for around $500. It still requires to be tested, but the NeoLife device performs the essential duties of a ventilator, mixing air and oxygen using intuitive switches, allowing to set the breathing rate and inspiratory time, and optionally operating as a standard continuous positive airway pressure (CPAP) machine. To come up with a device that performs all the vital functions of a regular ventilator but for 80 times less the cost, the students stripped down all the bells and whistles and focused on the most necessary components. That meant fitting a custom circuit board, an air pump, solenoid, pressure control valves and air flow valves into housing not much bigger than a shoebox. The students engineered the device entirely on their own—including the complicated printed circuit board that can be mass-produced at a small cost.

### Follow The Guidelines

New research has found that adhering to clinical guidelines for infants who were exposed to chorioamnionitis (CAM) and who had sterile blood cultures led to prolonged antibiotic therapy, longer length of stay, and more interventions. A retrospective data analysis published in Pediatrics notes that the Committee on the Fetus and Newborn (COFN) has recently changed their guidelines on the basis of these findings, and now antibiotic therapy for neonates with abnormal laboratory findings and those born to mothers with CAM does not have to extend for longer than 72 hours. Researchers at Thomas Jefferson University/Nemours in Philadelphia, Pennsylvania examined the frequency of abnormal laboratory testing in term and late-preterm neonates born to mothers with CAM and assessed the effects of following recent COFN guidelines. Of 554 infants meeting inclusion criteria, 83 (14.9%) had an abnormal immature to total neutrophil ratio (>0.2), 121 (22%) had an abnormal C-reactive protein level (>1 mg/dL), and 153 (27.6%) had either or both of these abnormalities at 12 hours of age. Blood culture was positive in only 4 (0.7%) of the infants. A more specific test or tool is needed to guide therapy in asymptomatic neonates exposed to CAM and with sterile blood cultures, the researchers said. Of 134 infants (24.2%) who received prolonged antibiotic therapy, 112 (20.2%) were treated only because of abnormal laboratory findings. More than one fifth (21.6%; n = 120) underwent lumbar puncture. The take-home message for clinicians is that a large number of term and late-preterm infants exposed to CAM with sterile blood cultures will have abnormal laboratory data. By following recent COFN recommendations,

1 in 4 healthy infants exposed to CAM will be treated with prolonged antibiotic therapy, will be subjected to additional invasive procedures, and will have prolonged hospitalization.

### Girl Babies 'Winning'

New research from the Robinson Research Institute looks at why boys are more likely than girls to be born preterm or suffer from certain neonatal problems. The research has found that there are undeniable genetic and physiological differences between boys and girls that extend beyond just the development of their sexual characteristics, and that girls are "clearly winning in the battle for survival," with better outcomes for female babies for preterm birth, stillbirth, neonatal death, and other complications after birth, such as macrosomia (a baby that weighs more than 4-4.5kg or 8 pounds 13 ounces at birth). Male babies generally grow faster and bigger than females. Following the examination of 300 placenta samples, researchers found that over 140 genes were expressed differently across the samples from both genders, suggesting there is a distinct sex bias in the regulation of genes in the human placenta.

### Kentucky Targets Neonatal Abstinence Syndrome

The state of Kentucky's maternal and child health leaders have said they will be working together to address the rising number of infants born with neonatal abstinence syndrome, a condition caused by exposure to narcotics during pregnancy, with the initiative bringing together health officials, the March of Dimes and others. In 2000, fewer than 30 infants in Kentucky were diagnosed with NAS, but in 2013 the number of cases was more than 950. The cost of care for the babies has grown from $190 million in 2000 to $720 million in 2009. Kentucky spent $40 million in 2012. Results collected by the Kentucky Perinatal Quality Collaborative will provide information toward standardized treatments to improve the outcomes of mothers and children affected by NAS. Initially, the focus will be on interventions for hospitalized newborns with NAS, including medication and non-medical treatments. Gov. Steve Beshear said he has made improving the health and wellness of Kentucky's children, families and workforce one of his highest priorities, launching kyhealthnow as an aggressive and wide-ranging initiative to reduce incidents and deaths from Kentucky's dismal health rankings and habits.

### Herpes Passed On To Newborns

Preventing herpes simplex infections in newborns requires additional research to identify women who are unknowingly shedding the virus at delivery, according to Natalie O. White, DO, a neonatology fellow at Nationwide Children's Hospital. She said current guidance that focuses primarily on aggressive evaluation and treatment of asymptomatic newborns delivered to women with active genital herpes simplex virus (HSV) lesions at delivery will likely not prevent most neonatal HSV infections. White and colleagues, including Infectious Diseases in Children Editorial Board member Pablo J. Sanchez, MD, conducted a study to assess the utility of the current guidance on management of asymptomatic neonates born to mothers with active genital HSV lesions. They reviewed medical records of infants aged younger than 42 days who were diagnosed with HSV infection at either Children's Medical Center in from December 2002 to August 2013 and at Nationwide Children's Hospital from October 2001 to October 2013. A diagnosis of HSV disease was found in 101 neonates and data on the mother's infection status was available for 100 (95%) infants. The records revealed that 7% of mothers had genital HSV infection either before pregnancy (n=1), at delivery (n=2), or unknown time (n=4). HSV disease in these 100 infants was caused by HSV-2 in 57% of infants and HSV-1 in 43%; 14% were preterm (<37 weeks' gestation). One infant was born with congenital HSV-2 infection and subsequently died. Skin/eye/mouth disease was present in 38% (n=36) of 97 infants, followed by disseminated disease in 27% (n=26), and central nervous system disease (CNS) in 35% (n=35). Median age at presentation was 11 days.

### Midwife Studies Neonatal Deaths in Utah

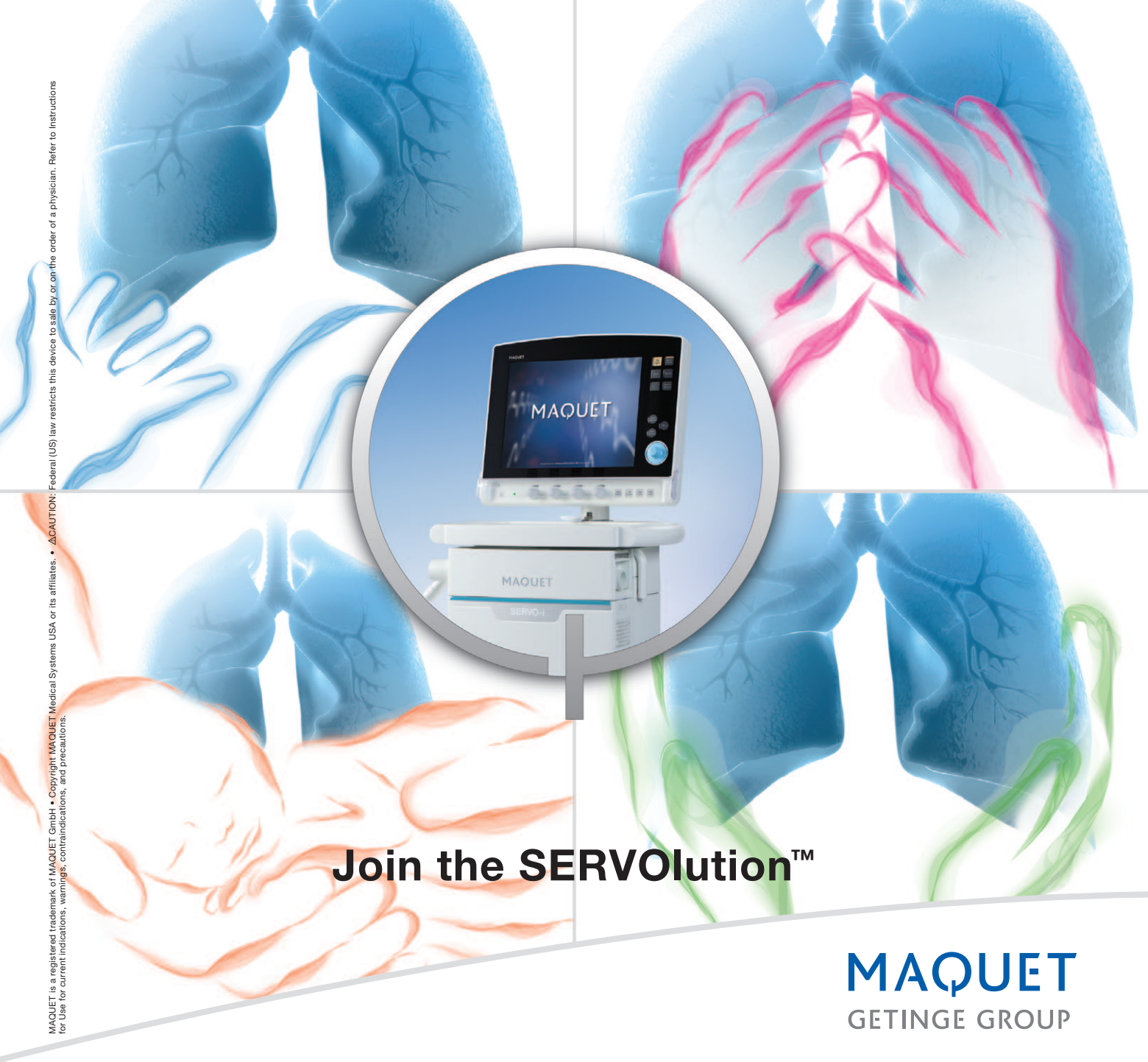
Donna Young, a Utah midwife used public sources, including obituaries and mortuary records, to document a surge in infant deaths in her town of Vernal to see if air pollution was causing a rise in neonatal deaths. The rate of neonatal mortality appears to have climbed from about average in 2010 to six times the national average in 2013, according to Utah Physicians for a Healthy Environment. In winter, pollution from drill rigs, wells and pipelines, as well as from nonindustrial sources, pools overhead, obstructing Vernal residents' view of the Uinta Mountains to the north. Now the local health department, with guidance from the state epidemiologist, is investigating whether poor birth outcomes are on the rise in Uintah County, which is experiencing a massive expansion in oil and gas development. The study will likely examine incidences of premature delivery and low birth weight, in addition to neonatal mortality, said state epidemiologist Sam LeFevre. Attempts will be made to account for risk factors, such as teen pregnancies and smoking during pregnancy, but the study won't attempt to establish causal connections. Information in this article first appeared in the Salt Lake Tribune.

### Bermuda Touts Midwives

Bermuda's high standards of healthcare meant that the island recorded no maternal or neonatal deaths in 2013, with a low prematurity rate of around 8% indicating women in Bermuda receive a high standard of prenatal care, according to Maternity and the Special Care Baby Unit clinical manager Janet Wheelan, who spoke ahead of King Edward VII Memorial Hospital midwives joining a worldwide campaign marking International Day of the Midwife. A hospital spokesman said that, while health statistics for pregnant woman and newborns in Bermuda are "very positive", more than 300,000 women and three million infants in other countries die annually from preventable complications during pregnancy and childbirth. The World Health Organization advises that midwives are key to achieving global reductions in maternal and newborn deaths and disabilities. Yet currently, there is a worldwide shortage of trained midwives and it is estimated another 350,000 midwives are needed, the hospital said.

### Neonatal Equipment Is Big Business

According to a new market report published by Transparency Market Research, the global prenatal, fetal and neonatal equipment market was valued at USD 5.89 billion in 2012 and is expected to grow at a CAGR of 6.1% from 2013 to 2019, to reach an estimated value of USD 8.92 billion in 2019. Prenatal, fetal and neonatal care is gaining importance every year with increasing awareness, demand and affordability for the latest, improved and technologically advanced equipment that are available worldwide. Premature birth has been one of the leading causes of neonatal death globally, that kills more than 1 million newborns every year. There are approximately 15 million premature babies born every year across the globe that require urgent medical attention



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and intensive care, which creates the demand for neonatal care equipment. Prenatal, fetal and neonatal care equipment are also required for numerous other applications, such as treatment for hypothermia, jaundice management, fetal and neonatal monitoring, respiratory assistance and others. New and technologically advanced equipment in the prenatal, fetal and neonatal equipment market will further provide growth opportunities to this market in future. The key factor responsible for the growth of the prenatal, fetal and neonatal equipment market is increasing incidences of premature births worldwide. Africa and Asia are the largest contributors towards the premature newborns, with Africa exhibiting highest incidence rate of premature births, which was 12% of the overall births in the region in 2005. However, the US exhibits the second-highest incidence rate of premature births with 10.6% of all births in the region in 2005.

### Gates Goes Low-Tech

Melinda Gates says holding and feeding a newborn the proper way can make the difference between whether it lives or dies. The co-chair of the Bill & Melinda Gates Foundation is promoting some low-tech approaches as part of a push to improve the survival of newborns, a problem she said has been neglected. In a speech to the World Health Assembly in Geneva, she outlined plans to urge global health officials as well as national governments to implement practices that will make deliveries safer and keep newborns alive. That will require competing for the attention of health ministers deluged with other pressing matters, from expanding HIV services to addressing a growing prevalence of noncommunicable diseases such as cancer and heart disease. Many newborn deaths, Gates said in an interview, can be prevented by simple, inexpensive measures—such as teaching women to breastfeed, which immediately gives a baby nutrients and hydration, and guards against infection, one of the biggest killers of newborns. Or "kangaroo care"—a technique in which the infant, particularly a preterm baby, is held against the mother's skin to keep it warm and regulate the heartbeat. Or applying chlorhexidine, an antiseptic, to the stump where an umbilical cord has been cut, to prevent infection. Every year, 2.9 million infants die in their first 28 days of life, while 2.6 million die in the last three months of pregnancy or during childbirth, according to United Nations data. Newborn deaths account for 44% of all deaths in children under 5 years old—a proportion that has grown since 1990 because vaccines, malaria bed nets, and other interventions have helped keep older children alive. The number of children under 5 who died dropped 47% between 1990 and 2012, to 6.6 million.

### 'Fun' Hearing Screener Available

Audiology Systems recently announced that the MADSEN OAE—a new otoacoustic emissions (OAE) hearing screener by Otometrics—is now available for sale in the United States. MADSEN Alpha OAE is specifically designed for use with a pediatric population. The handheld MADSEN Alpha OAE offers intuitive touch screen navigation to ensure a wide range of operators will quickly master its use. An on-board cartoon actually engages children during the test, a simple innovation resulting in less fidgeting, squirming and frustration, and more successful tests. According to David Adlin, screening products manager with Otometrics, the Alpha is a "game changer in hearing screening. Our customers tell us how simple the Alpha OAE is to use, and the video really engages the child—it's actually fun. Periodic hearing screening and early detection of hearing loss is so important. MADSEN Alpha OAE makes this important task easy for both kids and the test operator." MADSEN Alpha is available for immediate delivery through Audiology Systems.

### Support System Introduced

International Biomedical has introduced the Neo-Restraint, a fully adjustable close proximity support system. The Neo-Restraint is reusable and can be retrofitted to any Airborne transport incubator. It is intended to prevent an accidental fall of the newborn from the incubator. The innovative design allows for full access to the infant along with quick release features when necessary. Designed with input from clinicians and medical design teams, pressure points are eliminated by wrapping the safety straps around the infant for non-abrasive support. The Neo-Restraint is reusable and can be machine-washed. Each Neo-Restraint system comes with small, medium and large size options to accommodate infants from 1.1 pounds up to 13.0 pounds.

### Puffin Cleared

International Biomedical, a leader in infant care products, has obtained a 510(k) regulatory clearance from US Food and Drug Administration (FDA) for their Puffin Infant Resuscitator. The clearance adds to the company's growing and diverse line of products. Almost 15% of all neonates are in need of respiratory support at birth. For those affected newborns, the Puffin offers everything needed at their bedside: T-Piece or bag and mask ventilation, integrated suction which makes airway management less stressful and blended air/oxygen delivery along with monitored delivery of positive pressure breaths. The portable and compact design of the Puffin can be used to enable respiratory support in all perinatal areas of the hospital: labor and delivery room, well baby nursery, neonatal intensive care and the emergency room. Puffin can be easily mounted to bedside warmers and incubators or placed at bedside on its optional mobile stand.

### Placenta Benefits Studied

The placenta, once thought sterile, actually harbors a world of bacteria that may influence the course of pregnancy and help shape an infant's health and the bacterial makeup of its gut, a new study has found. The research is part of a broader scientific effort to explore the microbiome, the trillions of microbes—bacteria, viruses and fungi—that colonize the human body. Those organisms affect digestion, metabolism and an unknown array of biological processes, and may play a role in the development of obesity, diabetes and other illnesses. During pregnancy, the authors of the new study, including Baylor College of Medicine and Texas Children's Hospital in Houston, suspect, the wrong mix of bacteria in the placenta may contribute to premature births. Although the research is preliminary, it may help explain why periodontal disease and urinary infections in pregnant women are linked to an increased risk of premature birth. The findings also suggest a need for more studies on the effects of antibiotics taken during pregnancy.

### Iodine Promoted for Women

Pregnant and breastfeeding women should seek out prenatal supplements that contain iodine, an element that is crucial to healthy brain development and may be lacking in their diets, the American Academy of Pediatrics said in a report. Iodine consumption has fallen over the last few decades.

About one-third of pregnant women have a mild iodine deficiency, the report says. This is the AAP's first statement on iodine supplementation during pregnancy, which is already recommended by the American Thyroid Association and other groups. The AAP, which represents 60,000 pediatricians and pediatric specialists, says women should seek out supplements that contain at least 150 micrograms of iodide, a source of iodine easily absorbed by the body. That, combined with dietary intake, should bring iodine consumption to the recommended 220 micrograms for pregnant women or 290 micrograms for breast-feeding women, the daily amounts recommended by the Institute of Medicine. Good dietary sources of iodine include dairy products, seafood and iodized salt. The increase in mild iodine deficiency may have happened because of increased consumption of processed foods, which generally contain noniodized salt, said the report. Iodine is necessary to produce thyroid hormone, which in turn helps the brain develop.

### Health Benefits Found With Women's Groups

The World Health Organization has recommended an intervention developed and tested by partners in four countries and UCL researchers to improve maternal and newborn health. The intervention involves groups of women working together in a four-stage facilitated process: 1) Identifying problems during pregnancy, delivery, and post-partum; 2) Developing strategies to address these problems; 3) Implementing these strategies; and 4) Evaluating the strategies. A meta-analysis of research into Participatory Women's Groups was conducted by Dr Audrey Prost and others, largely from UCL's Institute for Global Health, and appeared in The Lancet in May 2013. The researchers analyzed data from seven cluster randomized controlled trials in Nepal, India, Bangladesh and Malawi. The analysis found that exposure to women's groups led to a 20% reduction in neonatal mortality. The researchers also found that in women's group initiatives that achieved high coverage—meaning that at least 30% of pregnant women participated—there was a 49% reduction in maternal mortality, and a 33% reduction in neonatal mortality. With high coverage it was estimated that the deaths of 36,600 women and 283,000 children could be prevented.

# NANN PREVIEW

## Creche

Booth 205

We will be having some fun with events to be won at Booth #205. Join the stampede! Let's hear you cheer! NANN 2014 is finally here! This is one booth you won't want to miss because if you do, you just might be on the "WANTED" list!

### What products do you plan to exhibit at NANN?

1) The Penguin Nutritional Warmer is the # 1 choice by doctors/ neonatologists and nurses for standardized nutritional warming of breast milk and formula.

2) Our micro-size, medical-grade, breast milk refrigerators which runs at less than 39 decibels, retains the cold air when opened and allows for quick cooling recovery every time it is opened.

Say goodbye to frost build-up and constant alarms!

3) The Penguin Custom Cart specifically customized and designed to maneuver in any medical environment, has the ability to run on battery for up to 24+ hours and allows for mobile refrigeration.

### What's new this year…?

The Penguin Enteral Warmer—specifically designed to warm breast milk and formula for long-term continuous feedings. * Not currently available for sale in the United States.

The Penguin Nightstand has lockable storage space for a family's belongings, a supply drawer; the quietest refrigerator on the market built into it and is ideal for very small patient rooms.

The Penguin Wi-Fi Reporting System that works in conjunction with any refrigeration system that requires 24-hour temperature monitoring in order to meet JCAHO, FDA and other accreditation entities requirements.

And…a surprise you just won't want to miss!

### What educational or training materials will be available?

Here at Creche we believe in catering to our customers, therefore we will have multiple educators on-site to give one-on-one or small group presentations at all times, along with having a little bit of fun mixed in! Educational materials will be available for those who want to take a hardcopy home with them or we can electronically send them information right to their email from the show!

### Why should our readers stop by your display?

Yee-ha! C'mon over to booth 205! Pull on yer boots, don yer best jeans, throw on yer hat and git here by any means. We're gonna be throwin' a party you won't want to miss! Rumor has it YOU are on our Hero's WANTED LIST as we celebrate you, the heroes who take care of our fragile, premature babies on a daily basis and save thousands of lives every year! So put on your "duds" and scoot on over to booth 205 as it is gonna be a "Wild Wild West" of a good time!

## Medela, Inc

Booth 410

Medela, Inc. is the market leader in hospital-grade breastpumps, research, and education. Medela's systems of innovative, evidence-based products help NICUs to increase human milk feedings.

Research shows that babies breastfeed in 2 phases:

- Stimulation Phase – when babies first go to breast, they suck fast and light to start milk flowing.
- Expression Phase – after milk flow or "let-down" starts, babies breastfeed with a slower, deeper suck, bringing out more milk faster.

Medela's Symphony Breastpump is the first breastpump with 2-Phase Expression technology, designed to mimic a baby's natural nursing rhythm and proven to achieve faster milk ejection and faster milk flow (when pumping at maximum comfort vacuum in the expression phase).

Medela's Symphony Preemie+ Breastpump is the first breastpump with clinically proven research that improves

the pumping efficiency and effectiveness for mothers with premature infants. The Symphony Preemie+ 1.0 program is designed to get pump dependent mothers started breastpumping. Its unique burst/pause pattern is clinically shown to help mothers of premature infants initiate milk flow as it mimics baby's first sucking pattern after birth.

Medela has combined both the Preemie+ 1.0 program and the standard 2.0 program onto one easy-to-use Preemie+ program card. This allows mothers of premature infants to use one pump to initiate and maintain her milk supply.

Medela's Waterless Milk Warmer safely, conveniently and effectively warms breastmilk to temperatures consistent with expressed human milk, thaws to refrigerated temperature and eliminates potential contamination risks associated with warming feeds in hospital tap water. This bedside unit accommodates breastmilk bottles and most containers up to 250 mL and syringes 1 mL up to 60 mL.

Both products will be on display at the 2014 NANN Conference.

On May 6th, 2014, Medela, Inc. acquired the enteral feeding assets of Acacia, Inc., a Brea, CA based company that designs and produces a line of high-quality neonatal feeding devices. Medela is devoted to enhancing mother and baby health through the life giving benefits of breastmilk. Our mission/destiny statement guides all that we do. It inspires us to find new ways to support mothers on their breastfeeding journey. The acquisition and partnership with Acacia Neonatal now allows Medela to further support NICU parents and professionals by providing an enteral feeding product line that delivers the crucial first drops of human milk at a time when every drop counts.

Medela also offers NICU Clinical Education Specialty Symposiums. Medela education programs are supported by research and delivered by leading experts. Medela has had a long commitment to supporting breastfeeding research and as a result works closely with some of the foremost experts in the field of human lactation research. These relationships strengthen the foundation of our evidence-based education programs, allowing us to bring cutting-edge research to professionals. Discount coupons for our education programs will be provided in our booth, during the NANN Conference.

We invite you to stop by Medela Booth #410 to learn more about our products and educational programs, and join us in welcoming Acacia Neonatal to the Medela family.

## Utah

Booth 228

**What products do you plan to exhibit at NANN?**
Utah Medical Products, Inc. (UTMD) plans to exhibit its specialty neonatal and pediatric devices. UTMD manufactures several developmentally friendly Gesco devices and kits, which are well recognized, including Umbili-Cath, Nutri-Cath, Nutri-Lok, PICC-Nate, Dialy-Nate and Uri-Cath. In addition, UTMD will showcase its Deltran Plus closed, customizable, needleless blood pressure monitoring and sampling system and its Disposa-Hood.

**What's new this year? Tell us about your latest products or future plans.**
UTMD, with its Nutri-Lok closed enteral feeding system, has been a pioneer in enteral feeding safety. Nutri-Lok syringes, feeding bags, extension sets and catheters securely lock to prevent disconnections while also preventing accidental misconnections with standard luer connectors. UTMD plans to continue developing products with a NICU focus, including a standardized small bore enteral-specific connector.

**What educational or training materials will be available?**
In addition to UTMD's knowledgeable clinical product specialists, videos, instructional presentations and brochures will be available at no-charge to UTMD's clinical partners.

**Tell us about any speakers or in-booth promotions.**
UTMD's clinical product specialists will be available to answer questions, provide in-servicing and to discuss new device opportunities.

**Why should our readers stop by your display?**
Readers will appreciate speaking with UTMD's knowledgeable representatives about the numerous devices they use everyday in their clinical practices. UTMD is happy to assist with product questions and is eager to discuss ways in which its current devices, or perhaps a new device, can improve patient care. If a clinician is interested in implementing a UTMD device, a UTMD representative can arrange for a no-charge sample to be sent.

# SPOTLIGHT ON VENTILATION

## Covidien

The new Puritan Bennett 980 ventilator helps enable patients to breathe more naturally through some of the most innovative breath delivery technology available. Our innovative user interface features a highly customizable display with intuitive screen navigation. The newly designed Puritan Bennett 980 ventilator provides a unique ventilator assurance feature and an integrated expiratory filtration system. Advanced synchrony tools help clinicians set the ventilator to adapt to their patients' unique needs and help provide the appropriate level of support throughout the breath. For more information about the Puritan Bennett 980 ventilator, please visit www.covidien.com/PB980.

**In this feature, Neonatal Intensive Care interviews Dignity Health-St. Rose Dominican, Siena Campus' Cynthia Duncan, RNC-NIC about the proper warming of human milk.**

## Introduction

Human milk is the gold standard for the human infant because it contains all the necessary components for baby's developmental needs. Feeding at the breast is ideal because mom's milk production is aligned with the changing nutritional requirements of the infant. Unfortunately, with compromised infants and preterm infants, feeding at the breast is not always immediately possible.

When this occurs, more often than not, the mother will pump her milk and bring it to the NICU for refrigeration or to be frozen for use at a later date. As clinicians and scientists we have the understanding that the biochemical reactions, which deliver all molecular components of mom's milk to the proper targets in baby's system are in fact temperature dependent, therefore, we must ensure our feedings are being given at body temperature when the baby cannot latch on to mom.

The questions that every NICU, PICU, and Birthing Center across the country should be asking themselves is, (regardless of whether you are warming a bottle, syringe, volufeeder or preparing an enteral feed) "what warming method am I using to warm this feeding (i.e. nutritional warming devices, cup of water, warm water bath, or steam) and what consequences could my current warming methodology have on my patient's feeding, thus what effect could it have on my patient for the short and long-term future?

**Neonatal Intensive Care:** We know that your hospital has stopped using the "cup and glove" method and purchased a nutritional warming system. Can you tell us, what product you selected and why you chose the one you did?
**Cynthia Duncan:** We chose the Penguin Warmer. This product was exclusively chosen after trials and research because we felt it was the best at warming the milk properly without harming any of the breast milk components.

**NIC:** One of the principal and fundamental precepts of medical ethics is "Primum non nocere" (first, do no harm.) Aside from evaluating the product and having your staff in-serviced, what steps did you and your staff personally take to ensure the product was safe, that you believe other facilities should look at doing, so they can see for themselves, why the Penguin Nutritional Warmer is simply the best in class solution for proper nutritional warming?
**CD:** We made sure that the breast milk was heated slowly without overheating and providing a uniform temperature throughout the milk.

**NIC:** Aside from the disposable liner that must be used with the Penguin Warmer, the Penguin is optimized for any make, model and size of breast milk bag, volufeed, syringes (up to 140ml) and bottles (both glass and plastic). As a Nurse Manager, do you like being able to use various brands of supplies from various companies such as those listed above and know that they all will work within your current warming system or would you rather have the unit optimized to one brand and all your feeding supplies come from that company only?
**CD:** I feel that being able to warm multiple size containers is optimal. Milk is received from the milk bank in various size glass containers and mom's pump their milk and place it in various containers which best fits their need whether it's syringes, bags or volufeeders.

**NIC:** Do you have one warmer at each bedside? If yes, what do you see as the benefit to having one warmer at each bedside? If no, how many beds do you have, and how many warmers do you have? If you do not have one at each bedside, does that impact your staff's workflow in a positive or negative manner?
**CD:** We currently do not have one at each bedside but I feel that most of the babies which need one have one available to them. As census grows we will be evaluating the need to purchase additional warmers.

**NIC:** How would you address a clinician who states: " I would prefer to use a "waterless" device because…(1) I don't have to make one trip to the sink to partially fill a liner one-time in a 12-hour shift and it's more convenient and (2) I don't have to put my feeding in a bag of water, when in fact the liner used with the Penguin isolates the feeding in a "waterless" environment?
**CD:** The water is only for thermal purpose. The bag with isolated pouch of water does not come in contact with the bottle you are going to touch afterward to feed the baby increasing chances of contamination. Having the water around the bottle isolates the milk to warm and maintain to ideal maternal temperature for a longer period of time in the event the bottle is not used immediately after warming. The warmer gently vibrates to mix the milk and fat preventing different temperature spots giving a better even temperature. Consistency is preferred for premature

Input on questions was provided by Heather Townsend, RMSR, Executive Director of Healthcare Design and Education of Creche Innovations. If you would like to participate in this feature, as a company or healthcare provider, please contact Steve Goldstein at s.gold4@verizon.net.

infants who continuously have to struggle with temperature regulation. Providing them with one less thing to regulate is best for them creating easier digestion and less of a shock to the system.

**NIC:** We know that the Penguin Warmer does not expose the feeding container or the milk to heat greater than body temperature; however, there are warmers out there that use excessive heat to warm feedings, whether it be a bottle warmer, hospital grade warmer or enteral feed warmer. We also know that exposing BPA-Free feeding containers or enteral feed tubing to high heat can leach chemicals from the plastic feeding container into the feeding the baby will consume. Do you agree or disagree with the following statement from D. Warrino, PhD, "immunogenic response to extractables from feeding containers will negatively impact thymus development and positive and negative T-Cell selection. Such unwanted chemicals can have serious consequences to the developing immune system." Does the Penguin Warmer help ensure safety of feedings within your NICU?
**CD:** The breakdown of BPA chemicals in plastic products occurs at elevated temperatures damaging the thymus is several studies. The Penguin Warmer regulates the warming process to avoid these extreme temperatures. Although we practice with the use of BPA-Free containers and feeding tubes we do not know what other chemicals could be released from the container plastic in extreme or inconsistent temperatures until studies are conducted. With the Penguin Warmer's controlled temperature to human body temperatures it provides safety for the neonatal population.

**NIC:** What else can you tell us about the Penguin Nutritional Warmer that you feel is important and bas been beneficial and would be valuable for other hospital physicians, NICU/PICU/ Birthing Managers, Lactation Consultants, Educators and the nursing staff to know?
**CD:** Sealed thermal bags avoid cross-contamination and the equipment is easy to keep clean. Warmer has no small crevasses, door or edges to collect germs. Just drop and go, wipe and go. Simple to use.

**In this feature, Neonatal Intensive Care interviews clinicians and healthcare providers about the actual application of specific products and therapies. Participating in the interview from Mercy Children's Hospital School of Respiratory Care is Dana Evans, MHA, RRT-NPS.**

**Neonatal Intensive Care:** Please tells us about yourself, your experience and the role you play in furthering training in the respiratory field.
**Dana Evans:** I am currently the Respiratory Care Manager at Mercy Children's Hospital-St. Louis, located in St. Louis, Missouri. The NICU is a 122-bed, level III unit. I have been an RRT for 13 years. I am very active within our professional organizations, currently serving as the Missouri Society for Respiratory Care (MSRC) Education Committee Chair and Delegate to the AARC House of Delegates (where I am the Student Mentoring Committee Co-Chair). I have previously served as the MSRC Vice President, Director at Large, Program Committee Chair and District Vice President.

**NIC:** We heard you recently received a pretty impressive honor from the AARC. Would you be able to tell us about it?
**DE:** I was honored to receive the Management Section Specialty Practitioner of the Year Award at the AARC International Congress in November 2013.

**NIC:** Over the past few years, it seems the protocols for treating neonates with RDS have evolved significantly—from prophylactic surfactant and mechanical ventilation to less invasive approaches. In your opinion, what factors have driven this evolution?
**DE:** This change is driven by emerging research regarding best practice for initial respiratory support and surfactant administration. Studies conducted prior 2008 indicated that prophylactic surfactant led to a decrease in pneumothoraces, pulmonary interstitial emphysema and bronchopulmonary dysplasia (BPD). However, those trials did not compare prophylactic surfactant to nCPAP. In trials that compare these two different techniques of support, the benefits of prophylactic surfactant are no longer evident. In fact, initial stabilization on nCPAP (with early rescue surfactant as needed) has been found superior to prophylactic surfactant.

**NIC:** The AAP Committee on Fetus and Newborn recently published two consensus statements on Respiratory Support and Surfactant Administration in Preterm Infants. What were your opinions on these statements?
**DE:** I was very pleased to see these consensus statements published. They do an excellent job summarizing recent evidence in neonatal respiratory care. Many institutions will likely begin moving toward this best practice now that the AAP has endorsed it in this manner.

**NIC:** The AAP statements strongly recommend the early use of nCPAP as a first line therapy for preterm infants, with subsequent surfactant administration in infants who require additional support. Does this reflect the protocols in your NICU?
**DE:** Absolutely. Non-invasive support (via t-piece resuscitator or anesthesia bag) is a primary component of our delivery room care for the premature infant.

**NIC:** Do you have a gestational age at which you'll try initial nCPAP vs. intubation?
**DE:** This varies somewhat, depending upon the severity of the neonates respiratory distress. In general, our physicians will attempt a non-invasive ventilation in those neonates 28-30 weeks gestational age or older and in those neonates under 28 weeks who demonstrate a positive response to initial CPAP therapy via T-Piece resuscitator/CPAP bag. Of those neonates who fail initial nCPAP, many of them are given surfactant using the INSURE method and successfully transition back to nCPAP or nasal NIV.

**NIC:** Of the infants initially stabilized on nCPAP, what percentage fail—requiring either surfactant or mechanical ventilation?
**DE:** The COIN trial found that 50% required surfactant and 46% required invasive mechanical ventilation (Morley CJ, Davis PG, Doyle LW, Brion LP, Hascoet JM, Carlin JB; COIN Trial Investigators. Nasal CPAP or intubation at birth for very preterm infants. N Engl J Med. 2008; 358(7):700–708). It is important to note that tthis study was conducted specifically evaluating neonates born between 25-28 weeks gestation. As one might expect, we find more success with nCPAP alone in neonates of greater gestational age (when compared to those who are extremely premature).

**NIC:** The AAP statement on surfactant replacement therapy strongly recommends a clinical strategy of intubation, surfactant administration and rapid extubation to nCPAP—otherwise known as INSURE. Is this method commonly used in your NICU?
**DE:** Yes. We commonly administer rescue surfactant using the INSURE technique, with the neonate ultimately on nCPAP or nasal NIV.

**NIC:** How quickly will you try to extubate these infants following a dose of surfactant?
**DE:** Immediately. In fact, we do not secure the endotracheal tube

Input on questions was provided by Jason Beyer of Chiesi USA. If you would like to participate in this feature, as a company or healthcare provider, please contact Steve Goldstein at s.gold4@verizon.net.

in neonates we intend to use the INSURE method on. Once the surfactant has been administered by the respiratory therapist, the care team observes the neonate for return to baseline (vital signs, chest wall rise, etc). When this has occurred (usually 1-5 minutes) the ETT is removed and the baby is placed on nCPAP or nasal NIV.

**NIC:** What are the benefits of the INSURE method?
**DE:** Anytime we can minimize the duration of invasive ventilation, it decreases the risk of pulmonary trauma and other complications associated with this type of support. Therefore, the most benefit comes from RAPID extubation to nCPAP or nasal NIV. Further trials are needed to fully understand the long term benefits of this strategy.

**NIC:** Looking back, I know things have changed substantially over the years, but has the adoption of early nCPAP and early selective surfactant administration via the INSURE method improved outcomes?
**DE:** Recent literature has shown that the incidence of BPD is higher in neonates who received prophylactic surfactant when compared to those initially stabilized on nCPAP. Dani, et al. found that the INSURE method reduces the need for invasive mechanical ventilation and the overall duration of respiratory support (Dani, C, Corsini, L, Poggi, C, Risk factors for intubation–surfactant–extubation (INSURE) failure and multiple INSURE strategy in preterm infants. Early Human Development. 2012; 88(1), s3-s4). More research is needed to determine the long term outcomes associated with the INSURE method.

**In this feature, Neonatal Intensive Care interviews clinicians and healthcare providers about the actual application of specific products and therapies. Participating in the interview is the Founder of Halo Innovations, Bill Schmid. In addition to designing safe sleep products for babies, Schmid has also developed a program that effectively teaches new parents about safe sleep for babies before they bring their baby home.**

**Neonatal Intensive Care:** How and when did you get interested in safe sleep for baby?
**Bill Schmid:** Aside from the interest you develop when you bring your first newborn home, it was really the loss of Haley, our firstborn, at 8 weeks due to SIDS that fueled my passion.

**NIC:** What lead you to believe that the hospital setting was the ideal place to introduce new parents to safe sleep education for their newborn?
**BS:** It's really the first place parents are exposed firsthand to how their baby is being cared for by professionals…the Labor and Delivery nurses. Seeing what those nurses do with your baby is even more important, and will stay with you longer, than what the nurses say you should do.

**NIC:** What impact has this program had since its inception?
**BS:** Over 1300 hospitals now model safe sleep in their birth centers or NICU's with the help of Halo Innovations. This translates to over 1.6 million babies and their parents.

**NIC:** How do you reach new parents at the hospital with this vital information?
**BS:** HALO has two programs that hospitals can use separately or in combination. The first is what we call our In-Hospital

program which is geared towards replacing loose blankets in the birth center or NICU with HALO SleepSack Swaddles. The hospital is given a free, annual allotment of Swaddles to use, launder, and re-use in lieu of receiving blankets to model Safe Sleep in a blanket-free environment. Our second program is our Take-Home program where the hospital can purchase the retail version of our Swaddle (at a steep discount) and have it embroidered with the hospital logo to give to parents when they leave the hospital.

**NIC:** Are hospitals eager and interested to implement the Safer Way to Sleep Program in the NICU?
**BS:** For the most part, absolutely! There have been several published studies along with the AAP's recommendations to model and teach Safe Sleep to parents starting a couple weeks prior to discharge from the NICU. Modeling is recognized as being particularly important in the NICU because parents have been exposed to many care practices during their baby's stay there that they should NOT use at home (unless prescribed by their doctor) such as using a hat, using multiple blankets to wrap or position the baby, prone sleep position, etc. A switch to safe sleep modeling (supine sleep position, no blankets, etc.) should begin a couple weeks before discharge.

**NIC:** Are there any additional benefits to the Safer Way to Sleep Program?
**BS:** In addition to the Safe Sleep education, it is also important for parents to understand the risks associated with swaddling their baby in a way that prevents their baby from being able to flex and abduct their legs and hips. This is critical to the healthy development of hips and the prevention of Developmental Dysplasia of the Hips (DDH). The Halo SleepSack Swaddle has the endorsement of the International Hip Dysplasia Institute because of the freedom it gives the infant to flex and abduct his legs.

**NIC:** What is your penetration among newborns in the US and Canada as of this year?
**BS:** Over 1300 hospitals participate in HALO's Safer Way to Sleep program reaching over 1.6 million infants and their families each year. The program continues to grow at a rapid pace.

**NIC:** Is there anything more you would like to do in the hospital setting to further reinforce safe sleep for baby?
**BS:** HALO is always looking for ways to make sleep safer for infants and the hospital environment is a great place to reach parents.

**NIC:** In addition to using safe sleep garments at home, what can parents do to ensure safe sleep for their newborn?
**BS:** The infant's sleep environment needs to be safe and free of loose bedding. It is important for baby to be close to Mom for soothing and breastfeeding, but in his own sleep space. HALO has just introduced a product that can address the issues of closeness and safety—the HALO Bassinest Swivel Sleeper! It gives Mom easy access to her newborn in a safe, proximate location.

**NIC:** Are there any barriers that prevent hospitals from participating and if so, what solutions do you offer for those institutions that might be hesitant to participate.
**BS:** Frankly the biggest barrier we're facing today isn't the clinical aspects of our Swaddle it is occasional resistance from some third-party laundries to process our Swaddles due to the zipper and Velcro found on certain styles. With the recent introduction of a snap-version and Velcro-free options, we expect even this occasional roadblock to disappear.

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Input on questions was provided by Tricia Knigge of Halo. If you would like to participate in this feature, as a company or healthcare provider, please contact Steve Goldstein at s.gold4@verizon.net.

# Curosurf: A Proven Fit for Today's Less Invasive RDS Treatment Protocols

Chris Campbell

A nightmare for any parent—or medical professionals, for that matter—is watching an infant struggle to breathe on his or her own.

When it comes to the treatment of Respiratory Distress Syndrome (RDS), everyone witnessing this struggle wants the same thing—a fast, effective treatment that will be as gentle on the infant as possible.

The use of antenatal steroids and surfactant replacement therapy have long been shown to reduce the severity of RDS and mortality.[5,9] It's just a matter of finding the best way to match these treatments with ventilation.[10]

While mechanical ventilation and surfactant have played a primary role in the past in providing respiratory support for premature infants at birth, many practitioners today are looking to avoid the potentially harmful effects of mechanical ventilation—a modality that may potentially injure the lung through too much volume, pressure and resulting inflammation.[1,5,10,16]

Many practitioners are routinely choosing more gentle ventilation techniques, such as nasal CPAP, for initial stabilization of infants at risk for RDS.[11-15] While this can be effective, clinical trials suggest about 50% of infants will require subsequent intubation and potential surfactant administration.[7,13-15]

In infants requiring surfactant, the American Academy of Pediatrics recommends administration using the early rescue INSURE technique—which stands for intubation, surfactant, and rapid extubation.[11,16]

In this technique, infants are generally extubated 5-10 minutes following surfactant administration.[1,3,5,8]

When implementing the INSURE technique, surfactant choice is an important piece of the puzzle. Strong consideration should be given to such factors as surfactant volume, onset and duration of action, single-dose success rates and supporting evidence.[1-8]

CUROSURF (poractant alfa) Intratracheal Suspension is indicated for the treatment (rescue) of Respiratory Distress Syndrome (RDS) in premature infants.[2] CUROSURF reduces mortality and pneumothoraces associated with RDS.[2,6,17]

CUROSURF's low volume for efficient administration provides an initial dose that delivers a higher surfactant concentration in a lower volume compared to other exogenous surfactants.[2,18-22] Low volume may improve tolerability and has the potential to reduce complications such as airway obstruction.[23,24]

Experts around the world have put CUROSURF to the test through extensive study in various clinical settings, such as different gestational ages, birth weights and re-dosing thresholds.

Across these studies, administering CUROSURF via the early rescue INSURE technique resulted in consistently high rates of single-dose success,[25-28] including 91% (Verder H, et al. 1999), 100% (Dani C, et al. 2004), 83% (Bohlin K, et al. 2007, retrospective), and 84% (Leone F, et al. 2013, retrospective).

Across studies, administering CUROSURF via the early rescue INSURE technique significantly reduced the need for subsequent MV vs. alternate methods.[25-28]

In all four studies, infants were extubated within approximately 5-10 minutes following surfactant administration.[25-28]

Here are some of what the experts concluded about administering CUROSURF via the early rescue INSURE technique in these four major studies.

1. Bohlin K, et al. 2007, retrospective, which targeted moderately preterm infants with RDS, gestational age 27 to 34 weeks, in Sweden, wrote that: "By implementing a treatment strategy of surfactant administration by transient intubation during nCPAP (INSURE), the need for MV in moderately preterm infants is effectively reduced. Furthermore, the INSURE approach resulted in a decreased requirement of surfactant re-treatment, which may be the effect of a more pronounced and sustained improvement in oxygenation compared with conventional surfactant treatment followed by MV…Surfactant treatment improved oxygenation in all subjects although the treatment response appeared to be augmented after INSURE…In conclusion, a treatment strategy of surfactant administration by transient intubation during nCPAP (INSURE) is a safe alternative to surfactant treatment followed by MV in moderately preterm infants. INSURE significantly reduces the need for MV with no adverse effects on the outcome. For smaller neonatal units, the INSURE approach is an option to administer surfactant earlier and effectively treat RDS, particularly in a care setting where transfer is needed for MV."

2. Dani C, et al. 2004 out of Florence, Italy, which studied preterm infants <30 weeks' gestation, wrote that "newborns in the SURF-NCPAP group were extubated quickly and then

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retreated with NCPAP, whereas newborns in the SURF-MV group were treated with MV after surfactant administration and then were weaned gradually from MV…We found that, among infants being treated with NCPAP for iRDS, the immediate reinstitution of NCPAP after surfactant administration was safe and beneficial, as evidenced by the decreased need for MV and the shorter requirement for respiratory support, compared with infants who received MV after surfactant treatment. This strategy contributed to reducing the need for surfactant treatment and decreasing the stays of our patients in the intensive care unit, thus decreasing neonatal intensive care unit costs."

3. Leone F, et al. 2013, retrospective out of Padua, Italy, which studied premature infants, wrote that "the efficacy was even superior in terms of response and sustained oxygenation, of need for a second dose of surfactant, of recourse to more aggressive methods of ventilatory support, and of the reduction of respiratory co-morbidities and mortality."

4. Verder H, et al. 1999 out of Denmark, which studied newborns of less than 30 weeks' gestation, wrote that "in the present study the success rate for avoiding mechanical ventilation was equal for boys and girls in both treatment groups. Therefore, we believe that nasal CPAP combined with surfactant is an effective treatment in both sexes… Early treatment with surfactant, administered during a short period of intubation to infants with a/APo2 decreasing to 0.36, is a cost-effective intervention (moderate use of surfactant, very few complications) that significantly reduces the subsequent need for mechanical ventilation."

## References


1 Bohlin K, Gudmundsdottir T, Katz-Salamon M, Jonsson B, Blennow M. Implementation of surfactant treatment during continuous positive airway pressure. Journal of Perinatology. 2007;27: 422-427.
2 CUROSURF (poractant alfa) Intratracheal Suspension prescribing information, Cornerstone Therapeutics Inc, September 2010.
3 Dani C, Bertini G, Pezzati M, Cecchi A, Caviglioli C, Rubaltelli FF. Early Extubation and Nasal Continuous Positive Airway Pressure After Surfactant Treatment for Respiratory Distress Syndrome Among Preterm Infants < 30 Weeks' Gestation. Pediatrics. 2004;113:e560-e563.
4 Dizdar EA, Sari FN, Aydemir C, Oguz SS, Erdeve O, Uras N, Dilmen U. A Randomized, Controlled Trial of Poractant Alfa versus Beractant in the Treatment of Preterm Infants with Respiratory Distress Syndrome. Am J Perinatol. 2012;29(2):95-100.
5 Leone F, Trevisanuto D, Cavallin F, Parotto M, Zanardo V. Efficacy of INSURE during nasal CPAP in preterm infants with respiratory distress syndrome. Minerva Pediatr. 2013;65(2):187-192.
6 Ramanathan R, Rasmussen MR, Gerstmann DR, et al. A randomized, multicenter masked comparison trial of poractant alfa (Curosurf) versus beractant (Survanta) in the treatment of respiratory distress syndrome in preterm infants. Am J Perinatol. 2004;21:109-119.
7 Sandri F, Plavka R, Ancora G, et al. Prophylactic or early selective surfactant combined with nCPAP in very preterm infants. Pediatrics. 2010;125:1402-1409.
8 Verder H, Albertsen P, Ebbesen F, Greisen G, Robertson B, Bertelsen A, et al. Nasal continuous positive airway pressure and early surfactant therapy for respiratory distress syndrome in newborns of less than 30 weeks' gestation. Pediatrics. 1999;103(2):1-6.
9 Fraser J, Walls M, McGuire W. Respiratory complications of preterm birth. BMJ. 2004;329(7472):962-965.
10 Pfister RH, Soll RF. Initial respiratory support of preterm infants: the role of CP7,9-15AP, the INSURE method, and noninvasive ventilation. Clin Perinatol. 2012;39(3):459-481.
11 Committee on Fetus and Newborn. Pediatrics. 2014;133: 171-174.
12 Polin RA, Carlo WA, Committee on Fetus and Newborn. Surfactant replacement therapty for preterm and term neonates with respiratory distress. Pediatrics. 2014;133(1):156-163.
13 Morley CJ, Davis PG, Doyle LW, Brion LP, Hascoet J-M, et al. Nasal CPAP or Intubation at Birth for Very Preterm Infants. N Engl J Med. 2008;358:700-8.
14 SUPPORT Study Group. Early CPAP versus Surfactant in extremely preterm infants. N Engl J Med. 2010;362:1970-1979.
15 Dunn MS, Kaempf J, de Klerk A, Reilly M, Howard D, Ferrelli K, et al. Randomized trial comparing 3 approaches to the initial respiratory management of preterm neonates. Pediatrics. 2011;128(5):e1069-e1078.
16 Stevens TP, Harrington EW, Blennow M, Soll RF. Early surfactant administration with brief ventilation vs. selective surfactant and continued mechanical ventilation for preterm infants with or at risk for respiratory distress syndrome. Cochrane Database Syst Rev. 2007;17(4):1-32.
17 Fujii AM, Patel SM, Allen R, et al. Poractant alfa and beractant treatment of very premature infants with respiratory distress syndrome. J Perinatol. 2010;30(10): 665-70.
18 Surfaxin (lucinactant) Intratracheal Suspension prescribing information. Discovery Laboratories, Inc. 2012.
19 Survanta (beractant) Intratracheal Suspension prescribing information, Abbott Laboratories, Inc, March 2009.
20 Infasurf (calfactant) Intratracheal Suspension prescribing information, ONY, Inc, June 2011.
21 Moya FR, Gadzinowski J, Bancalari E, Salinas V, Kopelman B, Bancalari A, et al. A multicenter, randomized, masked, comparison trial of lucinactant, colfosceril palmitate, and beractant for the prevention of respiratory distress syndrome among very preterm infants. Pediatrics. 2005;115(4):1018-1029.
22 Sinha KS, Lacaze-Masmonteil T, Soler AV, Wiswell TE, Gadzinowski J, Hajdu J, et al. A multicenter, randomized, controlled trial of lucinactant versus poractant alfa among very premature infants at high risk for respiratory distress syndrome. Pediatrics. 2005;115(4):1030-1038.
23 Wiseman LR, Bryson HM. Porcine-derived lung surfactant. A review of the therapeutic efficacy and clinical tolerability of a natural surfactant preparation (Curosurf) in neonatal respiratory distress syndrome. Drugs. 1994. 48(3):386-403.
24 Speer CP, Gefeller O, Groneck P, et al. Randomised clinical trial of two treatment regimens of natural surfactant preparations in neonatal respiratory distress syndrome. Arch Dis Child Fetal Neonatal Ed. 1995;72:F8-F13.
25 Dani C, Bertini G, Pezzati M, Cecchi A, Caviglioli C, Rubaltelli FF. Pediatrics. 2004;113:e560-e563.
26 Verder H, Albertsen P, Ebbesen F, et al. Pediatrics. 1999; 103:1-6.
27 Bohlin K, Gudmungsdottir T, Katz-Salamon M et al. J Perinatol. 2007;27:422-427.
28 Leone F, Trevisanuto D, Cavallin F, et al. Minerva Pediatr. 2013;65:187-192.

# Rapid Measurement of Human Milk Macronutrients in the Neonatal Intensive Care Unit: Accuracy and Precision of Fourier Transform Mid-Infrared Spectroscopy

Jennifer T Smilowitz, PhD, Deborah S Gho, Majid Mirmiran, MD, PhD, J Bruce German, PhD, and Mark A Underwood, MD

## Abstract

**Background:** Although it is well established that human milk varies widely in macronutrient content, it remains common for human milk fortification for premature infants to be based on historic mean values. As a result, those caring for premature infants often underestimate protein intake. Rapid precise measurement of human milk protein, fat, and lactose to allow individualized fortification has been proposed for decades but remains elusive due to technical challenges.

**Objective:** This study aimed to evaluate the accuracy and precision of a Fourier transform (FT) mid-infrared (IR) spectroscope in the neonatal intensive care unit to measure human milk fat, total protein, lactose, and calculated energy compared with standard chemical analyses.

**Methods:** One hundred sixteen breast milk samples across lactation stages from women who delivered at term (n = 69) and preterm (n = 5) were analyzed with the FT mid-IR spectroscope and with standard chemical methods. Ten of the samples were tested in replicate using the FT mid-IR spectroscope to determine repeatability.

**Results:** The agreement between the FT mid-IR spectroscope analysis and reference methods was high for protein and fat and moderate for lactose and energy. The intra-assay coefficients of variation for all outcomes were less than 3%.

**Conclusion:** The FT mid-IR spectroscope demonstrated high accuracy in measurement of total protein and fat of preterm and term milk with high precision.

## Background

Premature infants require substantially more protein than term infants to achieve growth comparable to normal intrauterine rates.[1] Extremely premature infants are particularly vulnerable,[2] with poor postnatal growth for weeks or months resulting in lifelong neurodevelopmental consequences.[3-5] Human milk is optimal for growth and protection against infection for term infants but is nutritionally inadequate for moderate and extremely premature infants.[6,7] Although premature milk delivers higher levels of protein and energy compared with term milk,[8] these levels are still unable to meet the metabolic demands of the premature infant. Premature infants who receive protein fortified infant formula have improved growth[5,9,10] but a higher incidence of late-onset sepsis and necrotizing enterocolitis (NEC) than those receiving human milk.[11-13]

To reduce the risk of late-onset sepsis and NEC while supporting growth and neurocognitive development, human milk is often fortified in the neonatal intensive care unit (NICU). One common method for protein fortification in the NICU is the addition of a standard amount of human milk fortifier powder to breast milk, assuming that all premature breast milk is similar in composition.[14] The poor growth with this approach,[15,16] the wide variation in macronutrient content between and within mothers,[17,18] and the tendency of clinicians to underestimate protein intake[19] are well documented. A second approach is to individualize human milk fortification based on either the infant's rate of growth or metabolic markers such as albumin or blood urea nitrogen. Individualized approaches are associated with higher protein intake and improved growth,[20] however, the studied methods require the infant to "fall behind" prior to increasing protein supplementation.

The ideal solution, analysis of human milk within the NICU with individualized fortification of macronutrients, has been advocated for 2 decades[21] but remains elusive as standard analytical chemistry methods require instruments not readily adaptable to an ICU setting. Infrared (IR) spectroscopy analyzes differences in the absorbance of IR energy by specific chemical structures. Mid-IR analysis is the standard method for bovine milk component analysis, approved by the International Dairy Federation and the Association of Official Analytical Chemists.[22,23] Mid-IR analyzers are commonly used in the dairy industry in part because milk fat (carbon-hydrogen groups), protein (amide groups), and lactose (hydroxyl groups) absorb wavelengths in the mid-IR range at 3.48 µm, 6.465 µm, and 9.610 µm, respectively, and enable the calculation of total solids.[24] Previous IR spectroscopy analyses of the macronutrient content of human milk have used filter-based mid-IR and near IR (NIR).[25-28] Filter-based mid-IR spectroscopy does not provide the full mid-IR spectrum, and with NIR, absorption bands are the overtones of the spectral bands residing in the mid-IR region, making quantitative calculations complex and calibration procedures laborious and not transferable from 1 instrument to another.[24,29]

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**Table 1.** Precision of the Estimated Limits of Agreement for the Macronutrient Composition Measured by the Fourier Transform Mid-Infrared Spectroscope and Reference Methods.

| Component | CI Mean Difference | Limits of Agreement (d – 2s) | Limits of Agreement (d + 2s) | CI of Limits of Agreement, Lower | CI of Limits of Agreement, Upper |
|---|---|---|---|---|---|
| True protein (n = 115), g/100 mL[a] | 0.07, 0.09 | –0.06 | 0.22 | –0.08, –0.04 | 0.20, 0.24 |
| True protein (n = 115), g/100 mL[b] | 0.08, 0.12 | –0.10 | 0.30 | –0.13, –0.07 | 0.27, 0.33 |
| True protein (n = 115), g/100 mL[c] | 0.10, 0.14 | –0.10 | 0.34 | –0.14, –0.06 | 0.30, 0.38 |
| True protein (n = 115), g/100 mL[d] | 0.05, 0.09 | –0.13 | 0.27 | –0.16, –0.10 | 0.24, 0.30 |
| Fat (n = 66), g/100 mL | –0.52, –0.40 | –0.96 | 0.04 | –1.1, –0.85 | –0.07, 0.15 |
| Lactose (n = 63), g/100 mL | –1.2, –0.99 | –2.0 | –0.24 | –2.1, –1.8 | –0.43, –0.05 |
| Calculated energy (n = 60), kcal/100 mL | –8.9, –7.5 | –13.8 | –2.6 | –15.0, –12.6 | –3.9, –1.4 |

Abbreviations: CI, confidence interval; NPN, nonprotein nitrogen.
[a]True protein calculated from NPN for each premature and term milk sample.
[b]True protein calculated from mean NPN for premature and term milk (29%).
[c]True protein calculated from mean NPN for term milk only (30%).
[d]True protein calculated from mean NPN for premature milk (26%).

Fourier transform (FT) mid-IR fulfills both of these criteria and thus represents an attractive tool for precise, accurate, and detailed analysis of human milk components.[24]

The aim of this study was to evaluate the accuracy and precision of a FT mid-IR spectroscope small enough and simple enough for daily use in a NICU to quantitate the concentration of human milk macronutrients over a wide range of gestational ages.

## Methods

### Samples

Human milk samples across various lactation stages (day 2 through day 368 postpartum) were acquired from women who delivered at term (>37 weeks gestation, 69 donors, 84 specimens) and preterm (26-36 weeks gestation, 5 donors, 32 specimens) enrolled in 2 different ongoing lactation studies at the University of California Davis. This study was approved by the University of California Davis Institutional Review Board.

### Mid-IR Analysis

The Delta Lactoscope FTIR Advanced Mid-IR Dairy Analyzer (Advanced Instruments, Norwood, Massachusetts, USA) was modified by the manufacturer to accommodate smaller sample volumes. The instrument was first calibrated for human milk total protein, fat, and lactose using undiluted and diluted (1:10, milk:deionized water) Raw Milk Component Calibration Standards (Eurofins DQCI, Mounds View, Minnesota, USA). The composition of these bovine calibrants was determined by the Kjeldahl method (total nitrogen multiplied by 6.25; protein for human milk),[30] the gravimetric Mojonnier method (fat), and HPLC (lactose) and by oven drying to constant weight (total solids).[31] To determine how the use of human milk calibration influences human milk component measurements, a subset of human milk samples from the study (n = 14) that spanned the range of protein, fat, and lactose of interest was used to recalibrate the instrument. Data from the remaining human milk samples (those not used to calibrate the instrument) were reanalyzed using this second human milk calibration.

### Sample Preparation

Samples ranging from 60 to 200 mL in volume were coldthawed overnight at 4°C. On each test day, 10 milk samples were fully thawed at room temperature for 2 hours and placed in a heated water bath until the sample reached 38°C. Each milk sample was vortexed at the maximum speed for 20 seconds to ensure the sample was evenly mixed. Subsequently, each milk sample was split into 2 aliquots. The first set of aliquots (undiluted milk) was shipped on ice packs overnight to Eurofins DQCI for milk component analysis using reference chemical methods.[31] The second set of aliquots was diluted to 1:10 with deionized water, heated to 38°C, revortexed at the maximum speed for 20 seconds, and analyzed by FT mid-IR spectroscopy using the spectroscope in the UC Davis Children's Hospital NICU. All methodological comparisons made in this report are between diluted human milk samples (1:10, milk:deionized water) measured by the FT mid-IR spectroscope and paired undiluted human milk samples analyzed by reference methods. Diluted human term milk samples were measured in replicates (4-11x depending on available sample volume) by the FT mid-IR spectroscope to determine the intra-assay coefficient of variation. Data were provided as g/100 mL and described as weight percentage in this report.

### Analysis of Human Milk Macronutrients by Reference Chemical Methods

Human milk total protein (total nitrogen), nonprotein nitrogen (NPN), true protein, fat, and lactose were analyzed by Eurofins DQCI. Total nitrogen and NPN were measured in duplicate by the Kjeldahl method and true protein was calculated by subtracting the NPN from the total nitrogen content in each sample[31] and then multiplying by the conversion factor 6.25 for human milk protein.[30] The measured NPN of each sample measured by Eurofins DQCI was used to adjust the total protein concentration measured by the spectroscope to obtain its calculated true protein concentrations. Since we were interested in the clinical utility of the spectroscope, true protein was also calculated using the mean NPN for all of the milk samples, and separately using the mean NPN for preterm and term milk. The fat concentration of each human milk sample was measured in duplicate gravimetrically using the Mojonnier method.[31] The lactose concentration of each human milk sample was measured in duplicate by HPLC according to the established method for measuring components in dairy products.[31] The mean of each duplicate measurement for true protein, fat, and lactose was used in the final data set (absolute differences between duplicate measurements were low). Total digestible energy provided by each sample was calculated as the sum of energy contributed by each component per 100 mL. Energy derived from true protein and lactose was determined by multiplying the number of grams of each component per 100 mL by a factor of 4 kcal/gram, and

**Table 2.** Human Milk Composition Measured by the Fourier Transform Mid-Infrared Spectroscope and Reference Methods.

| Component | Spectroscope | | Reference | | Mean Difference (SD) |
|---|---|---|---|---|---|
| | Mean (SD) | Range | Mean (SD) | Range | |
| Fat (n = 66), % as g/100 mL[a] | 3.6 (1.1) | 1.1–6.6 | 3.2 (1.2) | 0.75–6.5 | –0.46 (0.25) |
| Fat preterm (n = 32)[a] | 3.6 (1.3) | 1.1–6.6 | 3.1 (1.4) | 0.75–6.5 | –0.49 (0.21) |
| Fat term (n = 34)[a] | 3.7 (1.0) | 1.6–6.4 | 3.3 (1.0) | 1.2–6.0 | –0.43 (0.28) |
| True protein (n = 115), % as g/100 mL[a] | 0.88 (0.39) | 0.29–2.0 | 0.96 (0.38) | 0.41–1.9 | 0.08 (0.07) |
| True protein preterm (n = 32)[a] | 1.1 (0.41) | 0.62–2.0 | 1.2 (0.38) | 0.74–1.9 | 0.08 (0.08) |
| True protein term (n = 83)[a] | 0.78 (0.34) | 0.29–1.9 | 0.86 (0.34) | 0.41–1.9 | 0.08 (0.07) |
| Lactose (n = 63), % as g/100 mL | 7.3 (0.35) | 6.5–8.2 | 6.2 (0.47) | 5.0–7.3 | –1.1 (0.43) |
| Lactose preterm (n = 28)[a] | 7.2 (0.25) | 6.6–7.6 | 6.1 (0.39) | 5.2–6.9 | –1.1 (0.51) |
| Lactose term (n = 35)[a] | 7.4 (0.39) | 6.5–8.2 | 6.3 (0.52) | 5.0–7.3 | –1.1 (0.36) |
| Calculated energy (n = 60), kcal/100 mL | 65.4 (9.8) | 43.2–90.1 | 57.2 (10.6) | 33.8–83.1 | –8.2 (2.8) |

[a]$P < .0005$, paired-samples *t* test between Fourier transform mid-infrared spectroscope and reference methods.

energy derived from fat was determined by multiplying the number of grams of fat component per 100 mL by a factor of 9 kcal/gram.[32]

## Statistical Analyses

All statistical procedures were conducted using SPSS version 20.0 for Windows (SPSS, Chicago, Illinois, USA). Means ± standard deviations (SD) are reported for the 1:10 diluted human milk samples that were measured by the FT mid-IR spectroscope and undiluted human milk samples measured by the reference chemical methods and their mean difference ± SD (reference – FT mid-IR spectroscope). The mean values and their differences for the FT mid-IR spectroscope and reference methods were analyzed for normality and extreme values through the SPSS Explore procedure and were transformed appropriately. One case with values greater than 3 box lengths from the 75th and 25th percentiles was deemed an extreme outlier and removed from all analyses. Levene's statistic was computed to determine the equality of variance in the difference for each component (reference – FT mid-IR spectroscope) between preterm and term milk. Pairedsamples t test was used to determine significant differences between each milk component analyzed by the FT mid-IR spectroscope and matched reference method. Mann-Whitney U test was performed on the difference of each component (reference – FT mid-IR spectroscope) to determine if the method was influenced by the gestational age of the milk (preterm vs term). Significance was set at $\alpha < .05$.

To determine how much the FT mid-IR spectroscope measurements differed from reference methods, we calculated the limits of agreement and the precision of the estimated limits of agreement. Bland-Altman plots were generated to visualize

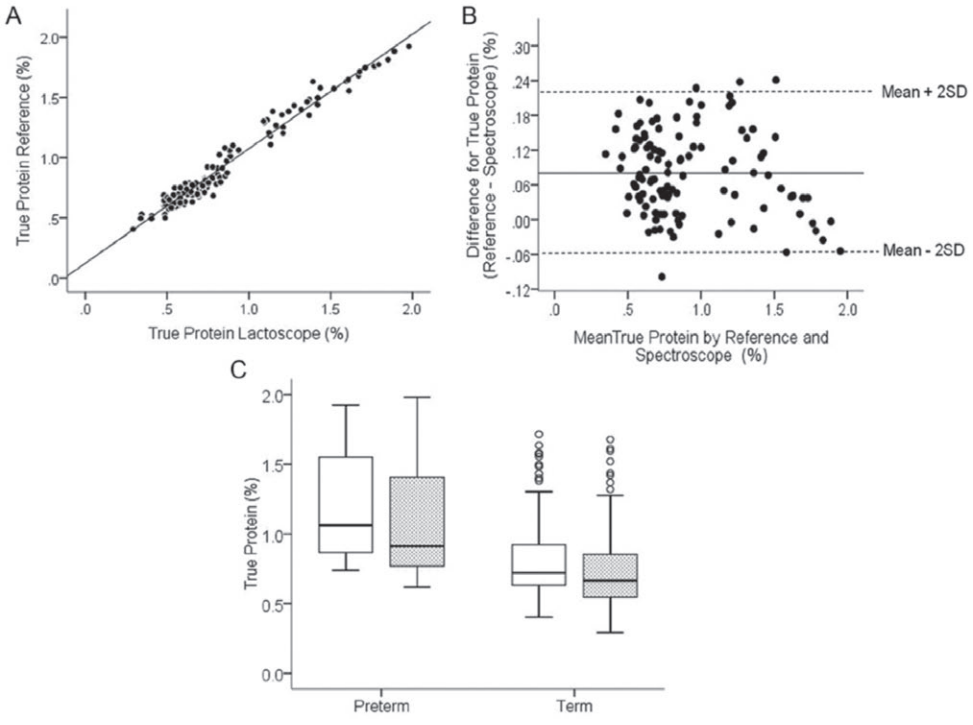


**Figure 1.** Human Milk Total Protein Concentration Measured by the Fourier Transform Mid-Infrared Spectroscope and the Reference Method.

A, Partial correlation between the 2 methods, r = 0.97, P < .0005. B, Bland-Altman plot displaying the mean differences and limits of agreement (n = 115). C, Distribution of total protein concentration in preterm (n = 32) and term (n = 83) milk measured by the Fourier transform mid-infrared spectroscope (white bars) and reference method (etched bars).

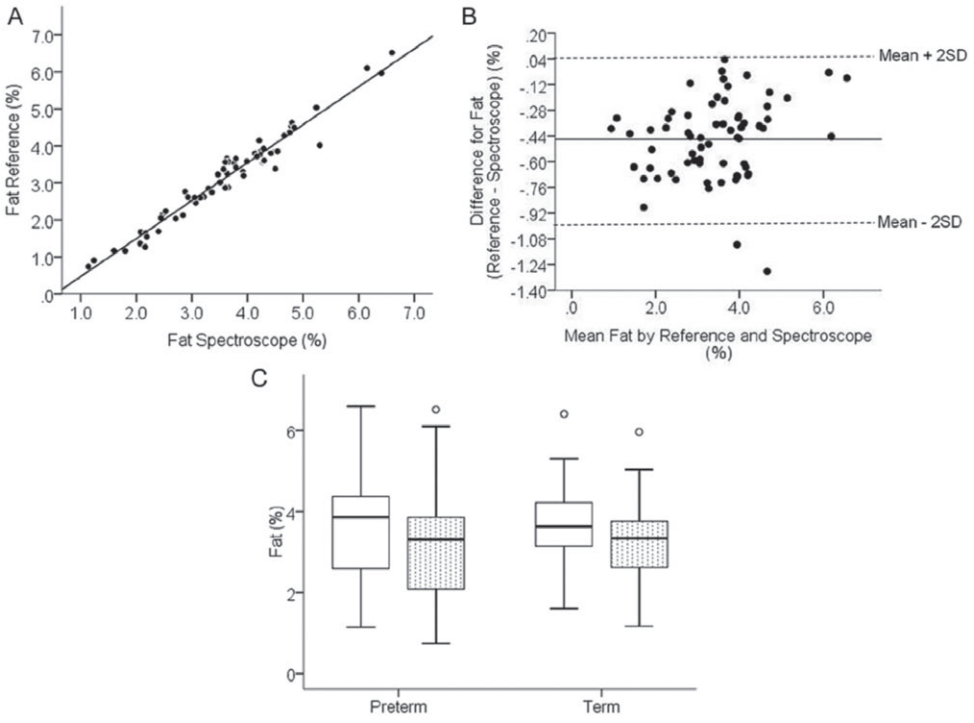


**Figure 2.** Human Milk Fat Concentration Measured by Fourier Transform Mid-Infrared Spectroscope and the Reference Method.

A, Partial correlation between the 2 methods, r = 0.98, P < .0005. B, Bland-Altman plot displaying the mean differences and limits of agreement (n = 66). C, Distribution of fat concentration in preterm (n = 32) and term (n = 34) milk measured by the Fourier transform mid-infrared spectroscope (white bars) and reference method (etched bars).

the limits of agreement.[33] Pearson product-moment correlation was used to determine the correlation between levels of human milk true protein, fat, and lactose measured by the FT mid-IR spectroscope as the dependent variables and reference methods as the independent variables. Box and whisker plots reflect medians, quartiles, and the 5th and 95th percentiles. To determine the reproducibility of the FT mid-IR spectroscope, repeated measurements for identical samples were analyzed for their means, SD, and coefficient of variation (CV). Intra-assay percentage CV was calculated as the mean CV for replicate measurements.

## Results

### Milk True Protein

The true protein concentration as determined by the FT mid-IR and reference methods for log-transformed data were highly correlated, r = 0.97, P < .0005 (Figure 1A). The Bland-Altman plot for human milk true protein demonstrates a high degree of agreement with the majority of the differences between the 2 methods plotted against their means within 2 SD from the mean difference (Figure 1B). The limits of agreement suggest that the FT mid-IR spectroscope measures human milk protein at concentrations 0.22% below to 0.06% above the reference method (Table 1). The mean difference for human milk true protein between the 2 methods was very small: 0.08% (Table 2). Based on the paired-samples t test on log-transformed data, the difference for human milk protein measured by the FT mid-IR spectroscope (diluted) and the reference method was statistically significant but quantitatively small (Table 2, Figure 1C).

The mean NPN measured by the reference method was 29% for all milk samples, 30% for term milk, and 26% for preterm milk. It is interesting that the variation for NPN was high with a range of 17% to 55% for all the milk samples and a narrower range for preterm milk (17%-39%). Despite the large variation in NPN, the limits of agreement were similar for true protein when calculated

**Table 3.** Reanalysis of Human Milk Composition by the Fourier Transform Mid-Infrared Spectroscope Using a Human Milk Calibration Model.

| Component | Reference | Spectroscope Human Calibration | Spectroscope Bovine Calibration | Difference Human (Reference – Spectroscope) | Difference Bovine (Reference – Spectroscope) |
|---|---|---|---|---|---|
| | Mean (SD) | | | | |
| Total protein (n = 101), g/100 mL[a] | 1.32 (0.43) | 1.22 (0.43) | 1.17 (0.43) | 0.10 (0.10) | 0.15 (0.10) |
| Fat (n = 55), g/100 mL | 3.06 (0.89) | 3.13 (0.98) | 3.53 (0.87) | –0.07 (0.43) | –0.47 (0.34) |
| Lactose (n = 53), g/100 mL | 6.16 (0.46) | 6.01 (0.42) | 7.27 (0.38) | –0.16 (0.45) | –1.11 (0.42) |
| Calculated energy, kcal/100 mL[b] | 57.5 | 57.1 | 65.5 | 0.4 | –8.0 |

[a]Total protein measured by the reference method was performed using Kjeldahl using 6.25 to convert total nitrogen to total protein.
[b]Energy was calculated from mean values for total protein, fat, and lactose listed in the table.

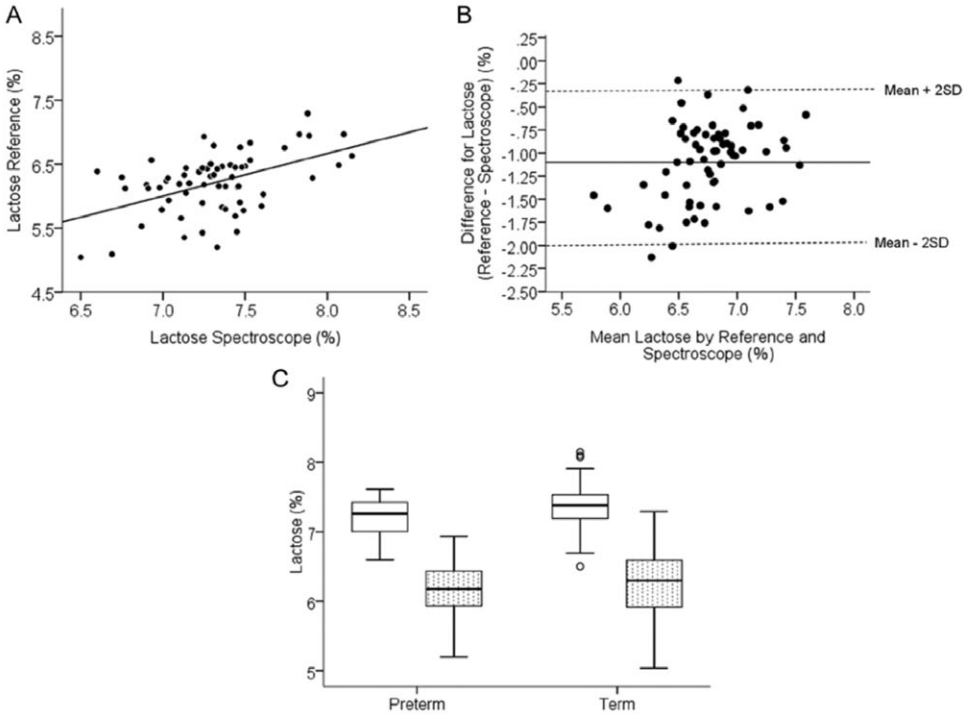


**Figure 3.** Human Milk Lactose Concentration Measured by the Fourier Transform Mid-Infrared Spectroscope and the Reference Method.

A, Partial correlation between the 2 methods, r = 0.49, P < .0005. B, Bland-Altman plot displaying the mean differences and limits of agreement (n = 63). C, Distribution of lactose concentration in preterm (n = 28) and term (n = 35) milk measured by the Fourier transform mid-infrared spectroscope (white bars) and reference method (etched bars).

by the different NPN ratios with the narrowest limits for 26% NPN compared with the widest range for 30% NPN (Table 1). In addition, the mean ± SD for the difference between the reference method and spectroscope for true protein was similar with each calculated % NPN: 0.10 ± 0.10 for 29% NPN, 0.12 ± 0.11 for 30% NPN, and 0.07 ± 0.10 for 26% NPN.

### Milk Fat

Human milk fat concentration measured by the FT mid-IR spectroscope and reference methods were highly correlated, r = 0.98, P < .0005 (Figure 2A), and showed high agreement (Figure 2B). The limits of agreement suggest that the FT mid-IR spectroscope measures human milk fat < 1% above the reference method (Table 1) with the mean difference between the 2 methods < 0.5% (Table 2). Based on the paired-samples *t* test, there was a significant difference for human milk fat concentration measured by the FT mid-IR spectroscope (diluted) and the reference method (undiluted) (Table 2, Figure 2C).

### Milk Lactose

Human milk lactose concentration measured by the FT mid-IR spectroscope and reference methods were moderately correlated, r = 0.49, P < .0005 (Figure 3A), with moderate agreement (Figure 3B). The limits of agreement suggest that the FT mid-IR spectroscope measures human milk lactose up to 2% above the reference method (Table 1). The mean difference for human milk lactose between the 2 methods was –1.1% (Table 2). Based on the paired-samples t test, there was a significant difference for human milk lactose concentration measured by the FT mid-IR spectroscope (diluted) and the reference method (undiluted) (Table 2, Figure 3C).

### Milk Energy

Calculated energy values for human milk measured by the FT mid-IR spectroscope and reference methods were highly correlated, r = 0.97, P < .0005 (Figure 4A), with moderate agreement (Figure 4B). The limits of agreement suggest that use of the FT mid-IR spectroscope to calculate energy levels may overestimate energy by up to 13.8 kcal/100 mL above the reference method (Table 1). The mean difference in calculated energy of human milk fat, total protein, and lactose concentrations measured by the 2 methods was –8.2 kcal (Table 2). Based on paired-samples t test, there was a significant difference for energy per 100 mL human milk sample calculated from the milk components (fat, protein, and lactose) in both preterm and term milk measured by the FT mid-IR spectroscope (diluted) and the reference method (undiluted) (Table 2, Figure 4C).

### Premature Versus Term Milk Measurements

The mean ranks for true protein, fat, lactose, and calculated energy measured by the FT mid-IR spectroscope and reference method were not statistically different between term and preterm milk samples (Mann-Whitney U test).

### Repeatability

Ten samples from women delivering at term were measured in replicate for fat, total protein, lactose, solids, and calculated energy using the FT mid-IR spectroscope (Supplemental Table 1). The intra-assay CVs were as follows: fat 2.4%, total protein 2.5%, lactose 1.6%, total solids 0.85%, and calculated energy 0.9%.

### Human Milk Calibration

Data from the 14 milk samples that encompassed the biological range for each milk component of interest (total protein, fat, and lactose) were used to calibrate the instrument, thereby adjusting the slope and y-intercept for each component. When data for total protein, fat, and lactose were reanalyzed for all

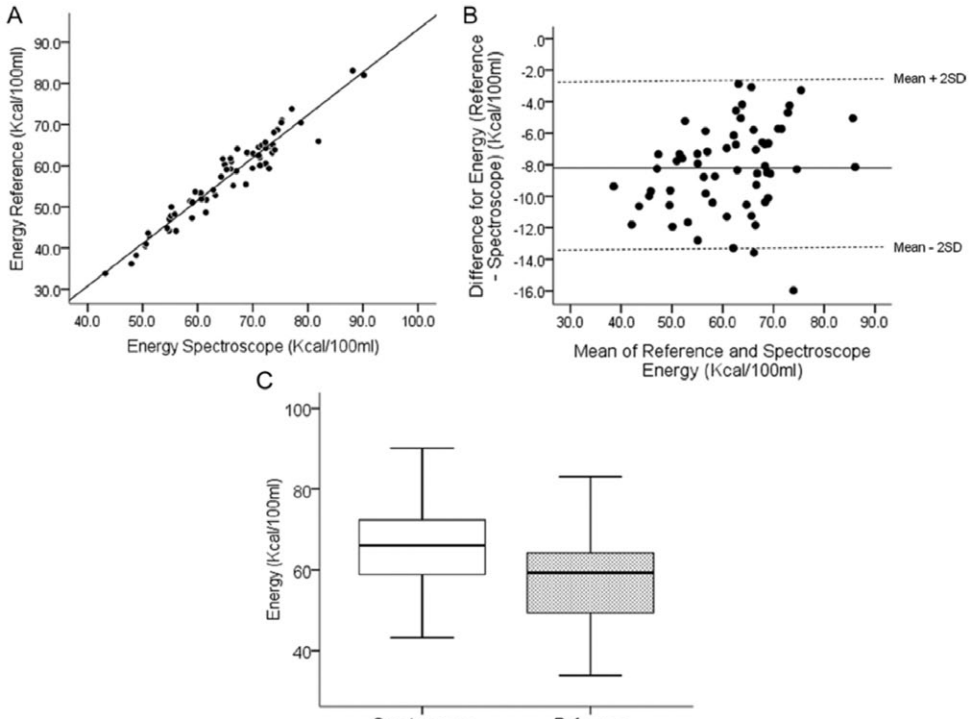


**Figure 4.** Human Milk Energy Calculated from Fat, Total Protein, and Lactose Measured by the Fourier Transform Mid-Infrared Spectroscope and the Reference Method (n = 60).

A, Partial correlation between the 2 methods, r = 0.97, P < .0005. B, Bland-Altman plot displaying the mean differences and limits of agreement. C, Distribution of calculated energy represented in the box plots for the sample set.

samples (excluding the 14 calibrants) by the spectroscope using a human milk calibration model, the values for total protein slightly improved. However, for human milk fat and lactose, the difference between the reference method and spectroscope values greatly improved, reaching values close to zero (Table 3).

## Discussion

Accepting the reference methods as the "gold standard," we found the FT mid-IR spectroscope to be most accurate in measuring protein content with high agreement between the 2 methods. The difference between the means was statistically significant, but of small magnitude and not likely to be clinically relevant. One of the challenges of rapid measurement of human milk protein is choosing the appropriate calibration standards. Bovine milk calibrants are readily available but have low NPN. Human milk calibrants are technically challenging in a clinical study. We adopted 3 approaches to address this challenge: correction of each sample based on its measured NPN, correction of each sample based on various mean NPN values, and calibration with human milk. Our data differ from reports from small clinical studies that found similar NPN concentrations between individual or pooled preterm and term milk.[6,34,35] Yet, the NPN measured in our study is close to the range of 17% to 27.7% observed for term and preterm milk.34 Human milk NPN concentration is highly variable and influenced by the lactation stage (higher in mature milk) and maternal diet.[34,37,38] Despite the large range for NPN, the limits of agreement were acceptable with each of the 3 methods. In practice, protein measured with the spectroscope and then adjusted based on a standard mean NPN should be acceptable for clinical purposes.

The overestimation of energy content by the FT mid-IR spectroscope is large enough to be clinically relevant and raises concerns about the use of this instrument to estimate total energy of human milk. When the spectroscope was recalibrated with data from a subset of human milk samples, total protein measurements changed only slightly whereas fat, lactose, and total energy were reduced to similar concentrations to the reference methods. The measurements likely improved with the human milk calibration because the 14 calibrants used were all within the reference range for each component measured in the samples. The use of bovine milk as calibration standards is limited to the natural range of bovine milk component concentrations. For example, lactose concentrations in the bovine milk standards used in this study ranged from 0.48% to 4.94%, which did not encompass the maximum concentration of human milk lactose measured by HPLC (7.3%). This suggests that human milk standards or supplemented bovine milk standards may improve the accuracy of this approach. It is unfortunate that human milk standards are not currently commercially available.

The rapid accurate determination of human milk macronutrient content would be of great benefit in the care of small premature infants, allowing individualized fortification based on intake rather than clinical or biochemical outcomes. We chose to test the FT mid-IR spectroscope for measuring human milk macronutrients based on its established accuracy and precision in the dairy industry39 and for its theoretical advantages over previously studied methods. First, the FT capability measures a very broad spectrum, enabling measurement of all molecules in the mid-IR range (unlike filter-based mid-IR spectrometers that measure a limited set of milk components). For example, the FT mid-IR spectroscope is able to measure total free fatty acids, citric acids, and casein and may be useful in the future for rapid determination of clinically relevant complex milk components of clinical interest that absorb within the full mid-IR spectrum such as human milk oligosaccharides (HMOs), lactoferrin, free fatty

acids, growth factors, and immunoglobulins. Furthermore, the raw data spectra can be saved for future analysis of molecules of interest without reanalyzing the samples. Second, because the organic bonds of milk molecules absorb in the mid-IR region, mid-IR is the standard method for bovine milk component analysis, approved by the International Dairy Federation and the Association of Official Analytical Chemists.[22,23] Third, the FT mid-IR spectroscope can be recalibrated on site and is not restricted to factory calibration. Calibration on site supports the future analysis of other milk components that absorb in the mid-IR bandwidth range. Fourth, for this study, the FT mid-IR spectroscope was modified to analyze a specimen of human milk as small as 1 mL (diluted to 10 mL); this is of particular importance in the NICU population given the often limited volume of human milk available.

Some of the discrepancy between the 2 measurements for lactose concentration appears to be related to the inclusion of non-lactose carbohydrates (eg, HMOs) in the FT mid-IR spectroscope measurements. The absolute concentration of HMOs in human milk varies across lactation and decreases from about 23 g/L in colostrum to 7 g/L in mature milk,[40,41] whereas bovine milk contains very low amounts of these bioactive oligosaccharides (micromolar range).[42] Human milk oligosaccharides shape the gut microbiota of the neonate and protect against adherence and invasion by pathogens.[43] It is important that there is wide variation in composition of HMOs in milk from mothers delivering prematurely,[44] which may explain some of the increased susceptibility in premature infants to intestinal dysbiosis and associated diseases like sepsis and NEC. When analyzed by the mid-IR spectroscope, purified HMOs were found to absorb in the same mid-IR spectral region as lactose (data not shown). Since the reference HPLC method used in this study does not measure HMOs (data not shown), it is likely that the higher lactose levels measured by the FT mid-IR spectroscope were a result of absorbing terminal or core lactose moieties of HMOs. Human milk oligosaccharides are easily identified by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy, but these technologies are not easily adapted in the clinical setting. One benefit to using Fourier transform mid-IR is the potential to develop algorithms that can adjust calculated HMO concentrations from the lactose measured for greater accuracy. Furthermore, with the use of mass spectrometry and NMR spectroscopy as validation tools, the potential of the mid-IR spectroscope to rapidly quantitate HMOs may have future clinical value.

The current study differs from previous comparisons in that they used filter-based mid-IR,[25,27,28] or NIR spectroscopy,[26] calibrated the mid-IR using only human milk calibrants,[25] and compared IR results with standard chemical analyses using correlations[27,28] rather than limits of agreement (the gold standard for validating new methods).[33]

Values generated from 2 different methods may differ in scale, influencing the limits of agreement but not the correlation, that is, data may have good correlation but poor agreement. For example, calculated energy for human milk was highly correlated between the 2 methods, yet their limits of agreement were fairly wide (–13.8 to –2.6 kcal/100 mL) such that energy intake by a premature infant consuming 300 mL of milk per day could be overestimated by 45 calories by the FT mid-IR spectroscope according to the confidence interval of the lower limit (–15.0, –12.6).

## Conclusion

The accuracy and precision of the FT mid-IR spectroscope is high for the most important macronutrient, protein, with acceptable accuracy for clinical purposes. The instrument appears well suited for clinical trials of an individualized approach based on daily measurement of milk specimens with fortification to predetermined protein content. This approach may have particular value in the use of pasteurized donor human milk (which is generally low in protein).

## References


1 Hay WW, Thureen P. Protein for preterm infants: how much is needed? How much is enough? How much is too much? Pediatr Neonatol. 2010;51(4):198.
2 Ehrenkranz RA, Younes N, Lemons JA, et al. Longitudinal growth of hospitalized very low birth weight infants. Pediatrics. 1999;104(2):280-289.
3 Mikkola K, Ritari N, Tommiska V, et al. Neurodevelopmental outcome at 5 years of age of a national cohort of extremely low birth weight infants who were born in 1996-1997. Pediatrics. 2005;116(6):1391-1400.
4 Isaacs EB, Morley R, Lucas A. Early diet and general cognitive outcome at adolescence in children born at or below 30 weeks gestation. J Pediatr. 2009;155(2):229-234.
5 Premji S, Fenton T, Sauve R. Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane Database Syst Rev. 2006;(1):CD003959.
6 Lemons JA, Moye L, Hall D, Simmons M. Differences in the composition of preterm and term human milk during early lactation. Pediatr Res. 1982;16(2):113-117.
7 Underwood MA. Human milk for the premature infant. Pediatr Clin North Am. 2013;60(1):189.
8 Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clin Nutr. 2011;30(2):215-220.
9 Cooke R, Embleton N, Rigo J, Carrie A, Haschke F, Ziegler E. High protein pre-term infant formula: effect on nutrient balance, metabolic status and growth. Pediatr Res. 2006;59(2):265-270.
10 Pieltain C, De Curtis M, Gérard P, Rigo J. Weight gain composition in preterm infants with dual energy X-ray absorptiometry. Pediatr Res. 2001;49(1):120-124.
11 el-Mohandes AE, Picard MB, Simmens SJ, Keiser JF. Use of human milk in the intensive care nursery decreases the incidence of nosocomial sepsis. J Perinatol. 1997;17(2):130-134.
12 Boyd CA, Quigley MA, Brocklehurst P. Donor breast milk versus infant formula for preterm infants: systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed. 2007;92(3):F169-F175.
13 Hylander MA, Strobino DM, Dhanireddy R. Human milk feedings and infection among very low birth weight infants. Pediatrics. 1998;102(3):E38.
14 Arslanoglu S, Moro GE, Ziegler EE, The Wapm Working Group On Nutrition. Optimization of human milk fortification for preterm infants: new concepts and recommendations. J Perinat Med. 2010;38(3):233-238.
15 Henriksen C, Westerberg AC, Ronnestad A, et al. Growth and nutrient intake among very-low-birth-weight infants fed fortified human milk during hospitalisation. Br J Nutr. 2009;102(8):1179-1186.
16 Lucas A, Fewtrell MS, Morley R, et al. Randomized outcome trial of human milk fortification and developmental outcome in preterm infants. Am J Clin Nutr. 1996;64(2):142-151.

17 Gross SJ, Geller J, Tomarelli R. Composition of breast milk from mothers of preterm infants. Pediatrics. 1981;68(4):490.
18 Weber A, Loui A, Jochum F, Bührer C, Obladen M. Breast milk from mothers of very low birthweight infants: variability in fat and protein content. Acta Paediatr. 2001;90(7):772-775.
19 Arslanoglu S, Moro G, Ziegler E. Preterm infants fed fortified human milk receive less protein than they need. J Perinatol. 2009;29(7):489-492.
20 Arslanoglu S, Moro GE, Ziegler EE. Optimization of human milk fortification for preterm infants: new concepts and recommendations. J Perinat Med. 2010;38(3):233-238.
21 Polberger S, Lönnerdal B. Simple and rapid macronutrient analysis of human milk for individualized fortification: basis for improved nutritional management of very-low-birth-weight infants? J Pediatr Gastroenterol Nutr. 1993;17(3):283-290.
22 Lynch JM, Barbano DM, Schweisthal M, Fleming JR. Precalibration evaluation procedures for mid-infrared milk analyzers. J Dairy Sci. 2006;89(7):2761-2774.
23 Horwitz W. Official Methods of Analysis of AOAC International. Gaithersburg, MD: AOAC International; 2000.
24 Lefier D, Grappin R, Pochet S. Determination of fat, protein, and lactose in raw milk by Fourier transform infrared spectroscopy and by analysis with a conventional filter-based milk analyzer. J AOAC Int. 1996;79(3):711-717.
25 Casadio YS, Williams TM, Lai CT, Olsson SE, Hepworth AR, Hartmann PE. Evaluation of a mid-infrared analyzer for the determination of the macronutrient composition of human milk. J Hum Lact. 2010;26(4):376-383.
26 Corvaglia L, Battistini B, Paoletti V, Aceti A, Capretti MG, Faldella G. Near-infrared reflectance analysis to evaluate the nitrogen and fat content of human milk in neonatal intensive care units. Arch Dis Child Fetal Neonatal Ed. 2008;93(5): F372-F375.
27 Menjo A, Mizuno K, Murase M, et al. Bedside analysis of human milk for adjustable nutrition strategy. Acta Paediatr. 2009;98(2):380-384.
28 Michaelsen KF, Pedersen SB, Skafte L, Jæger P, Peitersen B. Infrared analysis for determining macronutrients in human milk. J Pediatr Gastroenterol Nutr. 1988;7(2):229-235.
29 Reeves J III. Use of near infrared reflectance spectroscopy. In: Farm Animal Metabolism and Nutrition. London, UK: CAB International; 2000:185-208.
30 Lönnerdal B. Nutritional and physiologic significance of human milk proteins. Am J Clin Nutr. 2003;77(6):1537S-1543S.
31 Wehr HM, Frank JF. Standard Methods for the Examination of Dairy Products. Washington, DC: American Public Health Association; 2004.
32 Dewey KG, Finley DA, Lönnerdal B. Breast milk volume and composition during late lactation (7-20 months). J Pediatr Gastroenterol Nutr. 1984;3(5):713-720.
33 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;327(8476):307-310.
34 Atkinson S, Anderson G, Bryan M. Human milk: comparison of the nitrogen composition in milk from mothers of premature and full-term infants. Am J Clin Nutr. 1980;33(4):811-815.
35 Butte NF, Garza C, Johnson CA, Smith EB, Nichols BL. Longitudinal changes in milk composition of mothers delivering preterm and term infants. Early Hum Dev. 1984;9(2):153-162.
36 Lönnerdal B, Forsum E, Gebre-Medhin M, Hambraeus L. Breast milk composition in Ethiopian and Swedish mothers. II. Lactose, nitrogen, and protein contents. Am J Clin Nutr. 1976;29(10):1134-1141.
37 Lönnerdal B, Forsum E, Hambraeus L. A longitudinal study of the protein, nitrogen, and lactose contents of human milk from Swedish well-nourished mothers. Am J Clin Nutr. 1976;29(10):1127-1133.
38 Motil K, Thotathuchery M, Bahar A, Montandon C. Marginal dietary protein restriction reduced nonprotein nitrogen, but not protein nitrogen, components of human milk. J Am Coll Nutr. 1995;14(2):184-191.
39 Horowitz JF, Klein S. Lipid metabolism during endurance exercise. Am J Clin Nutr. 2000;72(2):558S-563S.
40 Coppa GV, Gabrielli O, Pierani P, Catassi C, Carlucci A, Giorgi PL. Changes in carbohydrate composition in human milk over 4 months of lactation. Pediatrics. 1993;91(3):637-641.
41 Gabrielli O, Zampini L, Galeazzi T, et al. Preterm milk oligosaccharides during the first month of lactation. Pediatrics. 2011;128(6):e1520-e1531.
42 Gopal PK, Gill H. Oligosaccharides and glycoconjugates in bovine milk and colostrum. Br J Nutr. 2000;84(suppl 1):S69-S74.
43 Zivkovic AM, German JB, Lebrilla CB, Mills DA. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci U S A. 2011;108(suppl 1):4653-4658.
44 De Leoz MLA, Gaerlan SC, Strum JS, et al. Lacto-N-tetraose, fucosylation, and secretor status are highly variable in human milk oligosaccharides from women delivering preterm. J Proteome Res. 2012;11(9):4662-4672.

# Reducing Blindness Means Looking in the Right Places

Chris Campbell

When it comes to challenging accepted norms of treatment, sometimes it takes bold pioneers to open other people's eyes.

Take, for example, the condition of Retinopathy of Prematurity (ROP), which is found in a small, but significant number of premature babies. When caught early, most of these babies respond positively to treatment.

For a long time, binocular indirect ophthalmoscopy was the only officially accepted method for diagnosis of ROP.

Darius Moshfeghi, MD of Stanford University, and colleagues sought to change all that.

For the last 8.5 years, Moshfeghi has led the Stanford University Network for Diagnosis of Retinopathy of Prematurity (SUNDROP) telemedicine initiative, and he is involved in a number of universal newborn eye screening initiatives.

The idea is to use trained screeners at various locations to collect images of a newborn's eye using the RetCam wide-field digital imaging system (Clarity Medical Systems, Inc, Pleasanton, CA).

"Images are collected at six remote locations by trained staff and then forwarded to me. I review the images for every infant that is screened, and if I see evidence of treatment-warranted disease, I either travel to the local site or have the baby transferred to my NICU to perform a dilated ophthalmoscopic exam of the patient before commencing treatment," Moshfeghi told Neonatology Today.

This change of thinking produced amazing results, but many were skeptical when it was first introduced. People are skeptical no more, Moshfeghi said.

"There are several studies to investigate if multiple graders come to the same diagnosis after examining retinal images of premature babies, but we have never evaluated the sensitivity and specificity of a binocular indirect ophthalmoscopy examination. It seems glaringly obvious to me that an image captured by a camera, which can be reviewed as many times and by as many experts as necessary, is a better means of diagnosis than a single individual viewing pathology and taking notes," states Moshfeghi.

Diagnosis of ROP using wide-field digital imaging rather than indirect ophthalmoscopy has now been officially accepted, paving the way for expansion of SUNDROP and other telemedicine programs.

New software has drastically increased the security and efficiency of image transfer. Synchronization software enables files to be automatically uploaded to a shared server once the camera is plugged into the network. The software also allows side-by-side image comparison and optional patient demographics.

"I can efficiently evaluate the images right on the server, make my report and send it," Moshfeghi said. "Not only is it much faster, it also eliminates user error. Babies go blind not because we lack effective treatment for ROP, but because we are not looking at them. This is due to a number of reasons including: technical difficulties, name changes or similar names, and multiple appointments. Anything that can be done to automate the screening process helps to eliminate human error."

Five-year data from the program reported that 1022 eyes of 511 patients were screened, of which 15 had treatment-worthy pathology and underwent therapy. "In addition to the successful treatment of all patients identified as needing it, this telemedicine program had 100% sensitivity, 99.8% specificity, 93.8% positive predicative value, and 100% negative predictive value for detection of treatment-worthy ROP. In essence, one baby was recommended for referral and did not need treatment, and no babies that needed treatment were missed," reports Moshfeghi.

The next step is to expand the program, with more coverage for remote locations and universal eye screening for newborns—ideas the team is exploring.

There is no federal requirement for newborn screening, but in China, testing of over 16,000 babies has shown an incidence of congenital cataracts alone of 0.1%.

The team is conducting a validation study to figure out the most cost-efficient person who can provide effective screening, and the primary outcome is to identify a cohort that can reliably identify the presence or absence of disease with a 98% agreement level. A key secondary outcome would be diagnosis agreement.

Chris Campbell is the Senior Editor of Neonatal Intensive Care.

## References

1 Fierson WM: American Academy of Pediatrics on Ophthalmology. Pediatrics, 2013.
2 Fijalkowski N, Zheng LL, Henderson MT. Clinical Science, Stanford University Network for Diagnosis of Retinopathy of Prematurity.
3 American College of Medical Genetics Newborn Screening Expert Group.
4 Centers for Disease Control.
5 Nie WY, Wu HR, Qi YS, et al.
6 Li-Hong L. Universal Eye Screening in Healthy Neonates.

# Breastfeeding The Infant With A Cardiac Defect

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According to the American Heart Association (2012), the prevalence of congenital heart defects (CHD) is estimated to be 9 per 1000 live births. Approximately 3 per 1000 infants with CHD require catheter-based or surgical intervention early in life. Infants with congenital heart defects are at-risk for failure to thrive, or growth failure as a result of increased energy expenditure and inadequate feeding capabilities. Growth parameters are monitored frequently to evaluate the timing for surgical interventions. Once growth slows it is deemed necessary to consider correcting the defect to promote normal growth and development. Very little literature exists regarding infants with congenital heart defects and breastfeeding.

## Classification of CHD

Infants with CHD are often classified by the direction of blood flow through the intracardiac shunts (Woodward, C., 2011; American Heart Association, 2012; Atz, A, Feinstein, J, Jonas, R, Perry, S, and Wessel, D, 1999; Mellander, M, 2013). Those defects that cause blood to flow from left to right are referred to as acyanotic lesions, such as atrial septal defect (ASD). Blood flow shunted across the ASD normally goes in the direction from the left atrium to the right atrium. Oxygenated blood returning from the lungs to the left atrium shunts to the right atrium, and mixes with deoxygenated blood returning from the body, causing an increased volume load on the right ventricle. Most cases of ASD have normal oxygen saturations and do not display any symptoms.

Infants with right-to-left shunts have defects referred to as cyanotic lesions. Tetralogy of Fallot, also known as TOF, is the most common cyanotic congenital heart defect. Characteristics of TOF include a narrow right ventricular outflow, a ventricular septal defect, and an overriding aorta. Deoxygenated blood flow leaves the right ventricle and meets obstruction as it tries to flow into the pulmonary artery. As this blood flows, it follows the path of least resistance by shunting some blood across the ventricular septal defect (VSD), which is a right-to-left shunt, and then passes into the aorta having bypassed the lungs to obtain oxygen. According to varying degrees of obstruction, these infants portray different levels of cyanosis and deoxygenation.

Complex CHD are the most complex classification with mixed lesions. An example is single-ventricular defect such as hypoplastic left heart syndrome. Infants presenting with this particular condition will experience cyanosis and ventilation-perfusion mismatch. Blood returning to the heart from systemic circulation and blood returning from the lungs mixes before being pumped by a single ventricle to the systemic and pulmonary circulation. These infants require several surgeries to redirect blood flow. Disease complexity of CHD is based on survival rates (American Heart Association, 2012). Atrial and ventricular septal defects are considered to be simple defects with survival rates greater than twenty years. Defects considered to be in the range of moderate severity include coarctation of the aorta, atrioventricular septal defect, ventricular septal defect with comorbidities, and tetralogy of Fallot. Congenital heart defects of great complexity include a single ventricle, truncus arteriosus, and transposition of the great arteries. Because overall survival rates for CHD have increased, there have been more incidences of neurodevelopmental disabilities or delays.

## Screening Criteria

Newborns at greatest risk for morbidity and mortality are those discharged from the hospital with undiagnosed congenital heart disease compared to those who had a diagnosis before leaving the hospital (de-Wahl, G., et al, 2009). Pulse oximetry can be an easy, inexpensive method for universal screening of newborns for CHD prior to discharge. Pre and post ductal oxygen saturation measurements are taken by placing probes on the right hand and either foot. Readings of less than 95% for pre and post ductal oxygen saturation, or a difference between the two measurements of greater than 3% is considered a positive result, necessitating referral for an echocardiogram. According to de-Wahl, G, et al, (2009), routine pulse oximetry screening of newborns has a significantly higher rate of detection than physical examination alone.

The United States Health and Human Services (HHS) Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) recommended in September 2010 that congenital heart disease (CHD) become part of the newborn screening process (Kemper, A, Mahle, W, Martin, G, et al., 2011; Mellander, M, 2013). The reason for this recommendation was to identify newborns with structural heart defects usually associated with hypoxia in the newborn period that could have significant morbidity and mortality early in life. There are seven specific lesions considered as primary targets for screening. These lesions are: hypoplastic left heart syndrome, pulmonary atresia, tetralogy of Fallot, total anomalous pulmonary venous return, transposition of the great arteries, tricuspid atresia, and truncus arteriosus. According to Kemper et al. (2011), the results of newborn CHD screening should be communicated to

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the newborn's primary care providers to ensure that necessary follow-up takes place.

### Health Care Costs For Screening

Main costs of the screening program for CHD is related to staff time for conducting the screening and tracking results, staff time spent for communicating test results with parents, purchase and maintenance of screening equipment, including probes, adhesive wraps, and cleaning supplies, and costs associated with verifying screening results and treatment (Kemper et al., 2011). Cost estimates reported in 2011 ranged from $5.00 to $10.00 per infant. Estimated time spent by staff to conduct the screening have been noted to be < 1 minute, or 5 minutes, including communication with parents. Cost estimates compare similarly to the cost estimates for newborn hearing screening. These cost estimates for pulse oximetry far offset the costs of care for complications of circulatory collapse resulting from an undiagnosed CHD.

Other costs related to screening include delayed newborn discharges for repeat screening or diagnostic evaluation, which may not be reimbursed by insurances (Kemper et al, 2011). "Echocardiography is typically reimbursed well" according to Kemper et al, (2011). Further costs involved with CHD diagnosis are the costs for transport of newborns to hospitals with pediatric cardiology services. Furthermore, there is no way to bill for pulse-oximetry monitoring according to Current Procedural Terminology (CPT) codes for pulse oximetry which are not accompanied by a diagnostic code for a pulmonary disease associated with hypoxia. Newborn hospital-based screenings such as hearing screening are commonly not reimbursed separately when conducted by regular nursery staff, even if appropriate CPT codes are used. Though health care costs related to universal newborn pulse oximetry screening is quite low, it is important to inform policymakers of the cost benefits of early diagnosis of CHD compared to costs associated with later diagnosis.

### Neurodevelopmental Outcomes

In 2012, the American Heart Association (AHA) published a scientific statement on the neurodevelopmental outcomes in children with congenital heart disease as being at increased risk of developmental disability or delay. This scientific statement was developed in conjunction with the American Academy of Pediatrics (AAP) to enable providers within the medical home to offer optimal medical care of infants and children with CHD. Optimal medical care includes developmental surveillance, screening, evaluation and reevaluation throughout childhood to enhance earlier versus later interventions and therapies for significant developmental deficits. Prevalence and severity of developmental disabilities (DD) and delays increase with the complexity of CHD. Recent studies have shown a significantly increased risk for DD in the areas of intelligence, academic achievement, language, visual construction and perception, attention, executive functioning, fine and gross motor skills, and psychosocial maladjustment.

In infants and children with CHD, there can be altered cerebral blood flow both in utero and after birth that may impact subsequent brain development (AHA, 2012; Sarajuuri, A, Jokinen, E., Puosi, R., et al, 2010). The brain is less mature and more vulnerable at birth than suggested by gestational age. Fetal and neonatal periods are critical times for brain growth and maturation, myelination, and development of neural pathways, which can lead to an increased risk of DD. Comorbidities for the delay in brain maturation in CHD infants include prematurity and late preterm newborns born at 34 to 36 weeks gestation. All of these infants meet the criteria for early intervention programs. Early screenings and referrals to supportive therapies are essential steps for maximizing potential for overall infant development.

### Feeding Difficulties

Many infants with CHD have feeding difficulties and failure to thrive (Pierre, A, Khattra, P, Johnson, M et al., 2010). Having increased energy expenditure, increased metabolic rate, and inadequate caloric intake for growth causes malnutrition. Failure to thrive has been defined as weight less than the 5th percentile (Black, M, Dubowitz, H, Krishnakumar, A, & Starr, R, 2007). According to Jadcheria, S. et al. (2009), these infants may simply be breathing too fast for safe oral feedings. Feeding difficulties with breast or bottle feeding can cause coughing, choking, gagging, or congestion during feedings as indicative of swallowing dysfunction (Pierre, A, Khattra, P, Johnson, M, et al, 2010; Arvedson, J & Brodsky, L, 2002). Poor sucking, milk leaking from the mouth during oral feeds, or not taking full feeds may be related to poorly developed oral motor skills, inappropriate rate of milk flow, uncoordinated swallowing, high respiratory rate, fatigue, and other gastrointestinal issues. Premature infants share many of the same feeding problems seen in infants with CHD, and as a result, oral motor assessments and feeding interventions are similar (Coker-Bolt, P, Jarrard, C, Woodward, F, & Merrill, P, 2013). Typical intervention for malnutrition and poor feeding behavior often leads to the placement of a nasogastric feeding tube and late introduction of oral feeds, which can lead to complications such as delayed oral motor development, gastrointestinal reflux, and swallowing abnormalities.

### Breastfeeding Capabilities

Prevalence of breastfeeding among infants with cardiac defects is unknown, but likely to be lower than the general population related to the challenges of the illness, hospitalization, and lack of encouragement and support from health care providers (Lambert J. and Watters, N. (1998). Mothers of infants with CHD often are not encouraged to breastfeed by the assumption that breastfeeding requires more energy and demands more oxygenation than for bottle-feeding. Two studies have been conducted that support breastfeeding as the preferred infant feeding method for the already compromised infant. One study compared oxygen saturation levels (SaO2) as an indicator of cardiorespiratory effort during breast-versus-bottle feeding. Results showed SaO2 levels were higher and less variable during breastfeeding than SaO2 levels during bottle feedings in infants with congenital heart disease (Marino BL, O'Brien P, and LoRe H, 1995). Combs VL and Marino BL (1993) compared growth patterns of breast and bottle fed infants with cardiac defects and found that those infants who breastfed gained weight more quickly and had shorter hospital lengths of stay. According to these studies, breastfeeding is less strenuous for infants with CHD, despite having a higher risk for failure to thrive and malnutrition.

### Case Scenario

This is a 32-year-old mother who delivered a 7 lb. 6 oz. baby boy at 38 weeks gestation. Diagnosis of CHD had not been anticipated prior to his newborn screening for CHD, which took place an hour before their anticipated hospital discharge. Once the diagnosis of CHD was confirmed, and his status considered safe for discharge, his established feeding plan was that of partial

formula and expressed breast milk fed by bottle. This mother met with a lactation consultant in her home when her infant was 16 days old. She had breastfed her first child and planned to breastfeed this infant as well.

The lactation consultant assessed her as experiencing low milk supply, and educated her on the importance of efficient milk removal from breast with the use of an effective breast pump to establish an adequate milk supply. This first consultation included obtaining the infant's weight, and conducting pre- and post-feeding weights using a valid infant scale for measuring breast milk intake from breast. The infant weighed 8 lb. 1 oz. and his intake from breast while breastfeeding was 30 ml. Feeding behavior consisted of a weak suck, poor seal, and falling asleep readily at breast despite usual arousal techniques such as breast compression and skin-to-skin positioning. The lactation consultant taught mother how to perform oral motor exercises, such as lip stretch, gum massage, and sucking with the use of a pacifier (see Oral Motor Stimulation Protocol, Coker-Bolt, P. et al, 2013). Plan of care as implicated by findings from this consult was to maintain infant weight gain with the use of a bottle since intake at breast was not sufficient for ongoing weight gain. The lactation consultant encouraged the mother to perform ongoing oral motor exercises to promote her baby's ability for breastfeeding.

Ongoing weekly consultations took place for three weeks with measureable intake from breast increasing from 30 ml, to 32 ml, 72 ml, and 142 ml respectively to exclusively breastfeed. This mother was able to increase her milk supply with the use of the Medela Symphony breast pump, which allowed her to express 5 to 7 ounces after each breastfeeding. This infant was diagnosed with Tetralogy of Fallot. At 3 months of age he had surgery to patch a large VSD (ventricular septal defect), closed an ASD (atrial septal defect), clamp his PDA (patent ductus arteriosus), and then opened up his pulmonary artery above and below the valve to fix his pulmonary stenosis. During the surgery and hospitalization this mother pumped every 3 hours and expressed 6 ounces each time.

After surgery this infant would not take a bottle as preferred by his pediatric cardiologist in order to track intakes. This mother felt as though her infant would be able to more readily breastfeed, and the cardiologist made an exception to his routine care by allowing her to breastfeed earlier than usual. Mother reported this breastfeeding went very well because he latched on immediately and had a full feeding. But then this mother said her infant slowed down at breast due to his GI system still being somewhat paralyzed from the anesthesia, and possibly from the discomfort of all the lines, chest tube, etc. that he was still hooked up to. At this time he would only take a few sucks and be done according to mom. It wasn't until after the chest tube was removed, a glycerin suppository, and about two and a half to three days after surgery that he was able to eat more. Once he was feeling better he picked back up like before (eating about 15 minutes total from both sides every 2.5-3 hours). This infant has maintained his growth pattern at 15% on the growth chart to the satisfaction of his pediatric cardiologist by feeding directly on breast before and after cardiac surgery.

## Conclusion

Infants with CHD present with challenges for breastfeeding related to misinformation regarding energy expenditure, risk factors for malnutrition and failure to thrive, and neurodevelopmental disability or delay. The minimal cost of screening for CHD in newborns needs to be made a part of newborn screening in all states to prevent health care costs associated with extended hospital stays and hospital readmissions. The American Heart Association and the American Academy of Pediatrics support the need for ongoing evaluation for developmental disability or delay for infants and children with CHD. As these infants survive with improved surgical interventions, more research is needed, as well as clinical education for health care providers to promote infant feeding directly at breast.

## References


1 American Heart Association. (2012).Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association. http://circ.ahajournals.org/content/126/9/1143/ Accessed 7/5/14.

2 Arvedson, J & Brodsky, L (2002). Pediatric swallowing and feeding: assessment and management, 2nd ed. Albany, NY: Singular Publishing Group.

3 Atz, A, Feinstein, J, Jonas, R, Perry, S, and Wessel, D. (1999). Preoperative management of pulmonary venous hypertension in hypoplastic left heart syndrome with restrictive atrial septal defect. The American Journal of Cardiology, 83; 1224-1228.

4 Coker-Bolt, P, Jarrard, C, Woodard, F, & Merrill, P. (2013). The effects of oral motor stimulation on feeding behaviors of infants born with univentricle anatomy. Journal of Pediatric Nursing, 28, 64-71.

5 de-Wahl Granelli, A, Wennergren, M, Sandberg, K, Mellander, M, Bejlum C, Inganas, L, Eriksson, M, Segerdahl, N, Agren, A, Ekman-Joelsson, BM, Sunnegardh, J, Verdicchio, M, Ostman-Smith, I. (2009). Impact of pulse oximetry screening on the detection of duct dependent congenital heart disease: a Swedish prospective screening study of 39,821 newborns. BMJ, Jan 8: 338.

6 Jadcheria, SR, Vijayapal, AS, and Leuthner, S. (2009). Feeding abilities in neonates with congenital heart disease: a retrospective study. Journal of Perinatology, Feb: 29(2): 112-118.

7 Kemper, A, Mahle, W, Martin, G, Cooley, WC, Kumar P, Morrow, WR, Kelm, K, Pearson, G, Glidewell, J, Grosse, S, and Howell, RR (2011). Strategies for implementing screening for critical congenital heart disease. Pediatrics 128(5): e1259-67.

8 Lambert, J, and Watters, N. (1998). Breastfeeding the infant/child with a cardiac defect: an informal survey. J Hum Lact 14(2): 151-155.

9 Mellander, M. (2013). Diagnosis and management of life-threatening cardiac malformations in the newborn. Seminars in Fetal & Neonatal Medicine 18: 302-310.

10 Pierre, A, Khattra, P, Johnson, M, Cender, L, Manzano, S, and Holsti, L. (2010). Content validation of the infant malnutrition and feeding checklist for congenital heart disease: a tool to identify risk of malnutrition and feeding difficulties in infants with congenital heart disease. Journal of Pediatric Nursing, 25; 367-374.

11 Sarajuuri, A, Jokinen, E, Puosi, R, Mildh, L, Mattila, I, Lano, A, and Lonnqvist, T. (2010). Neurodevelopment in children with hypoplastic left heart syndrome. J Pediatr 157; 414-420.

12 Woodward, C. (2011). Keeping children with congenital heart disease healthy. Journal of Pediatric Health Care 25(6): 373-378.

# Screening For Critical Congenital Heart Disease Using Pulse Oximetry, Role of Education in Maximizing Knowledge and Compliance: A Quality Improvement Project

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## Abstract

Critical congenital heart disease (CCHD) is a leading cause of death and disability among children. CDC and the AAP recommends Pulse Oximetry screening as part of routine newborn care. In February 2012, decision was made to implement this program in the newborn nursery at University of Illinois at Chicago (UIC). Following discussions with the different stake-holders, a Pulse-Oximetry screening policy was drafted for the institution. The nurses received a 20 minute presentation explaining the pathophysiology of congenital heart disease, mechanism of pulse oximetry and advantages and disadvantages of the screen. They completed an anonymous pre test and post test questionnaire. Of the 69 nurses who participated, 15 were excluded for incomplete data. Average pre and post test scores were 53% and 74% respectively ($p < 0.01$). Between October 21, 2012 and December 2013, 2128 babies were discharged from the newborn nursery, 2101 babies were screened. Our compliance rate was 98.7%.

## Introduction

Congenital heart defects are the most common type of birth defect in the United States, affecting nearly 1% or about 40,000 births per year.[1,2] Critical Congenital Heart Disease (CCHD) requires surgical (or catheter) intervention, typically within the first year of life and accounts for 25% of this number.[3] In addition, CCHD accounts for nearly 30% of infant deaths due to birth defects which makes early detection invaluable.[4] If missed, these infants are at risk for cardiovascular collapse and death/ long term morbidity.[3]

Fewer than 50% of cases are identified prenatally. In addition, CCHD is challenging to diagnose by clinical exam as signs such as a murmur or femoral delay may not be apparent. Mild hypoxemia (80-95%) may not produce visible cyanosis in the immediate post natal period.

Pulse oximetry screening is a simple noninvasive test that quantifies hypoxemia. In recent clinical studies, pulse oximetry has demonstrated high specificity (99.9%) and moderate sensitivity (76.5%) to detect CCHD and a low false-positive rate (0.14%) when performed beyond 24hr after birth.[5,6] Critical congenital heart disease was added to the US recommended uniform screening panel for newborns in 2011.[7] CCHD screening using pulse oximetry compares favorably to other newborn screening programs in place.[8]

Per AAP recommendations, screening should be done no earlier than 24 hours after birth and prior to the newborn being discharged from the hospital or birthing center using disposable or reusable motion-tolerant pulse oximeters that report functional oxygen saturation and have been cleared by the FDA for use in newborns. Screening should be based on the recommended screening algorithm developed by the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (Figure 1);

> Any infant with a positive screen should have a diagnostic echocardiogram, which would involve an echocardiogram within the hospital or birthing center, transport to another institution for the procedure, or use of telemedicine for remote evaluation. The infant's pediatrician should be notified immediately and the infant might need to be seen by a cardiologist for follow-up.

In February 2012, decision was made to introduce this screening program for detection of CCHD using pulse oximetry in the newborn nursery of University of Illinois at Chicago. It wasfelt that educating the nurses and patient care staff who would be performing the screening, to the importance of the screen would aid in gaining their cooperation. This in turn would translate to better compliance.

## Methods

Once the decision to start the screening program was made, the project followed the plan, do, study, act methodology (PDSA). Several meetings were conducted among the various stakeholders which included mother baby unit staff, pediatric cardiology, neonatology and most importantly nursing leadership. This resulted in formulation of a pulse ox screening

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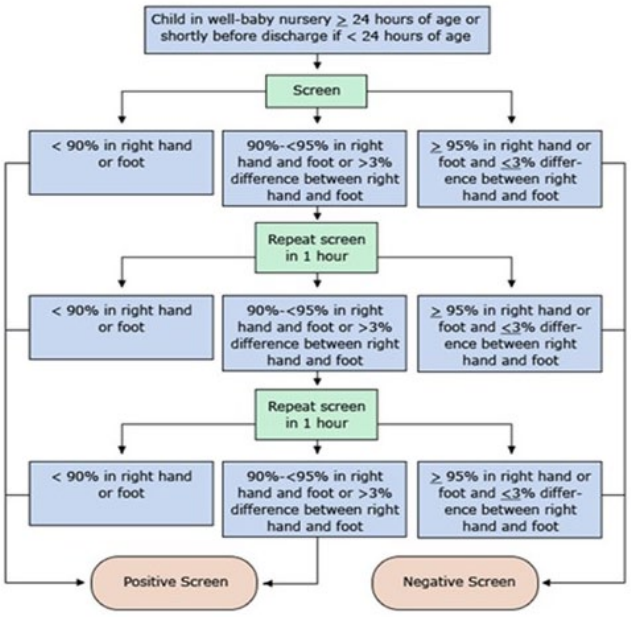



**Figure 1.** CDC Guidelines. A screen is considered positive if (1) any oxygen saruration measure is <90% (in the initial screen or in repeat screens); (2) oxygen saruration is <95% in the right hand and foot on three measures each separated by one hour; or (3) a >3% absolute difference exists in oxygen saturation between the right hand and foot on three measures each separated by one hour. Any screening that is ≥95% in the right hand or foot with a ≤3% absolute difference in oxygen saruration between the right hand or foot is considered a negative screen and screening would end. (Adapted with permission from Kemper A R et al. Pediatrics 2011;128:e1259-e1267).

policy for the institution for well newborns based on AAP recommendations.

A 20 minute power point presentation comprising pathophysiology of congenital heart disease, mechanism of pulse oximetry and usefulness of the screen was presented to the nursing staff by the pediatric residents. The screening algorithm with interpretation of the results was provided (Figure 1). The nurses completed an anonymous pre test and post test questionnaire (Figure 2).

**Screening for Critical Congenital Heart Disease using Pulse Oximetry**

1. Physical examination of the newborn accurately detects critical congenital heart disease.
[ ] True
[ ] False

2. What is the best time to check oxygen saturations in a newborn to screen for heart diseases?
[ ] in the delivery room
[ ] < 12 hours after birth
[ ] within 24 hours of birth
[ ] > 24 hours after birth

3. Which body site(s) should oxygen saturation be checked at for screening?
[ ] right hand only
[ ] right and left hand
[ ] right hand and either foot
[ ] right foot only

4. An infant's pulse oximeter screening results should be reported if
[ ] > 95% for both right hand and one foot and there is >3% difference between the two on 3 measures each separated by one hour
[ ] < 95% for both right hand and one foot or there is >3% difference between the two on 3 measures each separated by one hour
[ ] < 90% for either or both the right hand and one foot
[ ] all of the above

5. Which of the following do you think are critical congenital heart diseases? Check all that apply
[ ] Transposition of great arteries
[ ] Total anomalous pulmonary venous return
[ ] Hypoplastic left heart
[ ] Critical Coarctation of aorta
[ ] Ventricular septal defect

6. What do you understand by 'critical' congenital heart disease? Check all that apply
[ ] It requires surgery or catheter intervention during the first year of life
[ ] Most can be detected by physical examination alone
[ ] Missed or delayed diagnosis can be devastating
[ ] If diagnosed early, it can be managed with successful surgical repair or palliation

7. Is pulse-ox part of routine newborn screening per AAP recommendations?
[ ] Yes [ ]No

**Figure 2.** Questionnaire admisnitered to the nurses prior to and after the 20 minutes power point presentation.

New equipment including motion sensitive pulse oximeters and disposable pulse oximeter probes were purchased and procedural in-service training was conducted for the nurses. Screening was timed with PKU testing which was performed on neonates at or after 24 hours of life so as to effectively utilize available time of the nurses and more importantly to minimize risk of false positives from early screening.[5] The team worked with hospital IT department to be able to document the results of the screening part of the EMR. Decision was made to start the program with paper forms (Figure 3) and transition to electronic charting once the program was built into EMR (Figure 4). Literature was reviewed and a parent handout was created to be able to educate parents about the test and implications.

The CCHD screening program using pulse oximetry went live on Oct 15, 2012. Charge nurses in every shift were entrusted with troubleshooting issues that arose. Weekly statistics were collected by a core team of nurses to assess compliance to the procedure. CCHD screening was made one of the components of the biyearly competency that nurses undergo routinely to maintain quality and reinforce the correct technique of performing the screen. Audits were performed every 6 months to ensure compliance to the procedure and address areas of concern.

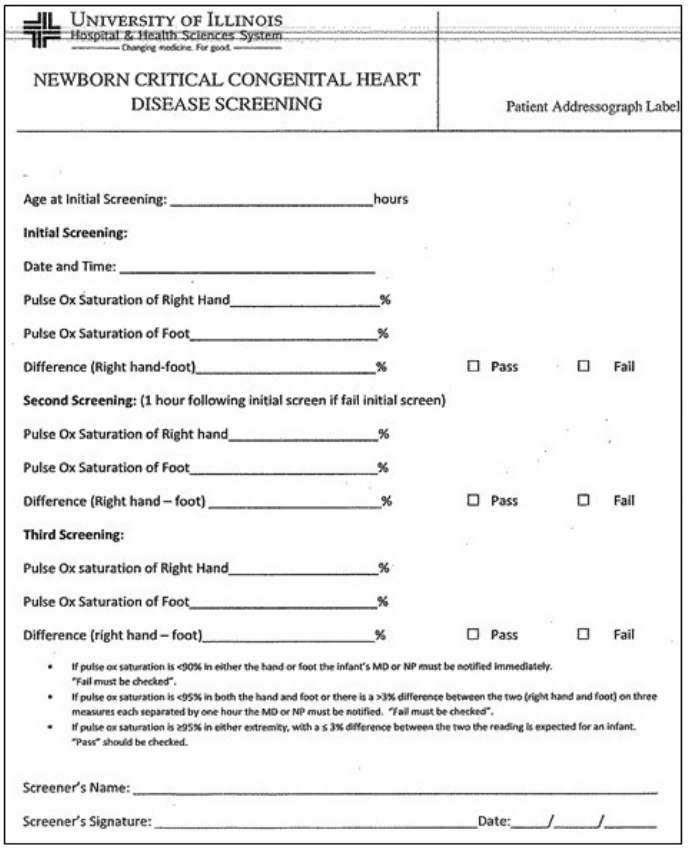
## Results

(I) The pre test and post test questionnaires were administered to 69 staff and flex nurses. 15 were excluded for incomplete information. Average pre test and post test scores were 53% and 74% respectively ($p < 0.01$) (Table 1). (II) Between October 15, 2012 and December 31, 2013, 2128 babies were discharged from the newborn nursery. 2101 babies were screened with no positive screens. Data were missing from 2 charts in Nov 2012. This was addressed and found to be a lapse as it was around the initial stages of the project. 22 babies were not screened as they were transferred to the NICU for further evaluation. In addition, there were 3 parent refusals (Table 2). (III) Our compliance rate was 98.7%.

## Discussion

The goal of this QI project was to set up a new screening program in a teaching hospital and more importantly sustaining compliance. Our project describes the steps involved in setting up and starting a new program in a clinical setting. It involved careful planning and discussion among the different stakeholders to establish a felt need for such a program. Implementation of such a program at this level is a team effort and the success depends on getting buy-in from all members of the team especially those who perform the procedure which in this case are the nurses.

The 1st PDSA cycle was aimed at laying the foundation for starting this program which involved reviewing the literature and the recommendations, meeting with the different stakeholders to assess challenges involved and feasibility. The most important step was getting



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NEWBORN CRITICAL CONGENITAL HEART DISEASE SCREENING

Patient Addressograph Label

Age at Initial Screening: _____hours

**Initial Screening:**

Date and Time: _____

Pulse Ox Saturation of Right Hand_____%

Pulse Ox Saturation of Foot_____%

Difference (Right hand-foot)_____% ☐ Pass ☐ Fail

**Second Screening:** (1 hour following initial screen if fail initial screen)

Pulse Ox Saturation of Right hand_____%

Pulse Ox Saturation of Foot_____%

Difference (Right hand – foot) _____% ☐ Pass ☐ Fail

**Third Screening:**

Pulse Ox saturation of Right Hand_____%

Pulse Ox Saturation of Foot_____%

Difference (right hand – foot)_____% ☐ Pass ☐ Fail

- If pulse ox saturation is <90% in either the hand or foot the infant's MD or NP must be notified immediately. "Fail must be checked".
- If pulse ox saturation is <95% in both the hand and foot or there is a >3% difference between the two (right hand and foot) on three measures each separated by one hour the MD or NP must be notified. "Fail must be checked".
- If pulse ox saturation is ≥95% in either extremity, with a ≤ 3% difference between the two the reading is expected for an infant. "Pass" should be checked.

Screener's Name: _____

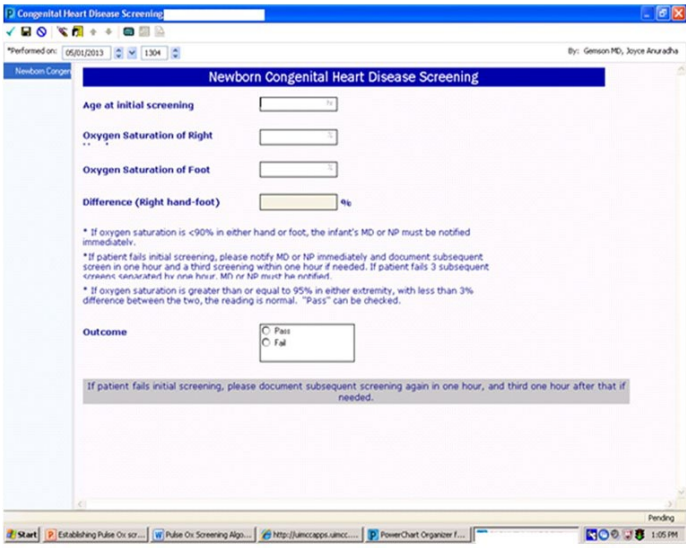
Screener's Signature: _____Date:____/_____/_____

**Figure 3.** Copy of the paper form used to record the results of the CCHD screening.

buy in from nursing leadership as well as nursing and patient care staff by improving their knowledge through the brief presentation and proved by the anonymous questionnaires.

The 2nd PDSA cycle was aimed at launching the program after ensuring appropriate infrastructure, recording results appropriately and maintaining compliance via various short term and long term measures using a core team of nurses (Table 3).

### Strengths of the program include:




1. Excellent teamwork especially the unconditional support from nursing leadership

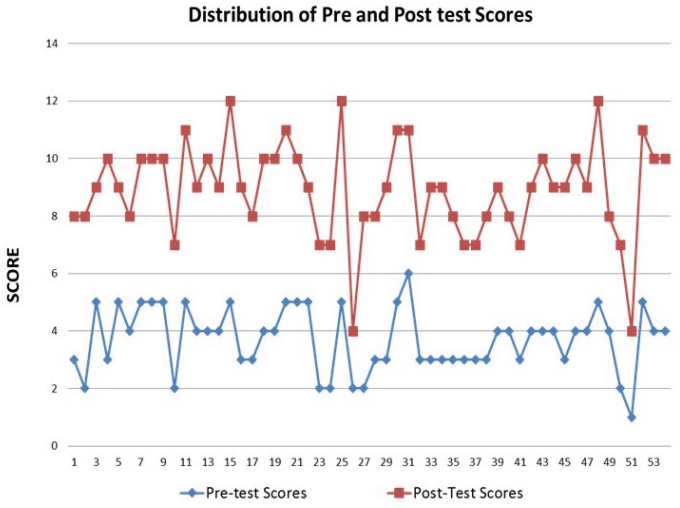



**Table 1.** Formatted line chart representing pre and post test scores from the nurses. X-axis representing the nurses included and Y-axis representing the scores obtained in the pre-test and post-test questionnaire.

| Month | Newborn Discharged | Screen performed/passed | Failed | Transfer to NICU | Refusal |
|---|---|---|---|---|---|
| Oct' 12 | 48 | 47 | | 1 | |
| November | 173 | 168 | | 3 | |
| December | 143 | 142 | | 0 | 1 |
| Jan' 13 | 146 | 146 | | | |
| February | 129 | 129 | | | |
| March | 130 | 129 | | 1 | |
| April | 124 | 124 | | 0 | |
| May | 173 | 168 | | 5 | |
| June | 131 | 130 | | 1 | |
| July | 142 | 141 | | 1 | |
| August | 142 | 141 | | 1 | |
| September | 172 | 172 | | 0 | |
| October | 154 | 153 | | 1 | |
| November | 161 | 156 | | 5 | |
| December | 160 | 155 | | 3 | 2 |
| | **2128** | **2101** | **0** | **22** | **3** |

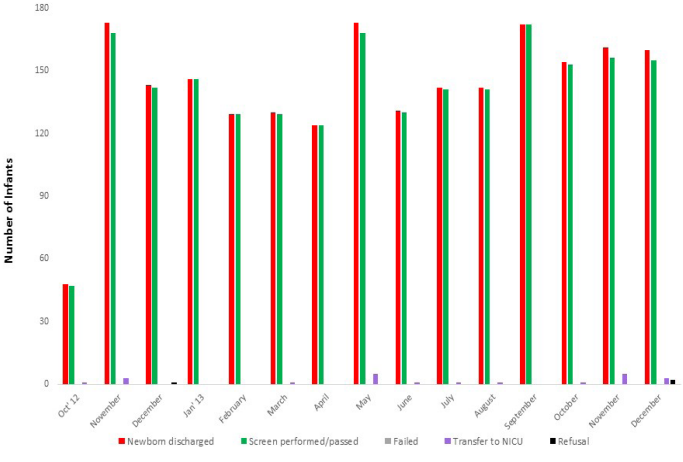



**Table 2.** Bar diagram with months on the X-axis and number of infants on the Y-axis. It represents a month by month comparison of the number of newborns discharged, the number of newborns who received the screening, those who passed/failed were missed.

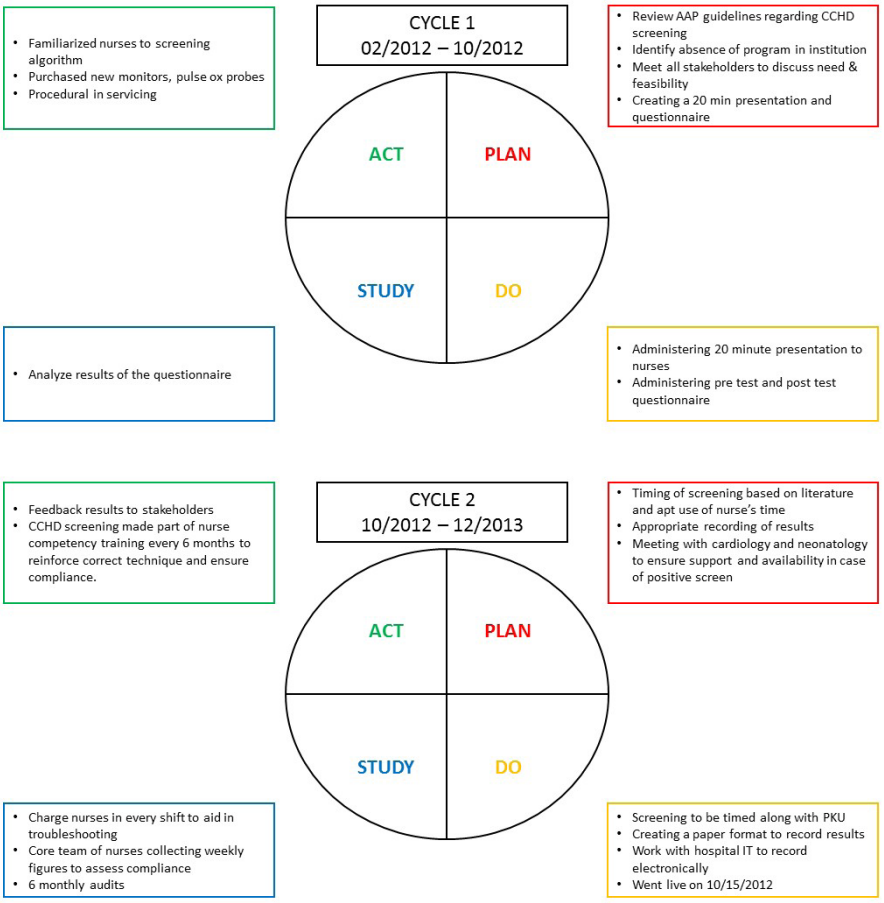



**Table 3.**

2. Setting realistic short term and long term goals for the team as well as the program.

### Challenges included:

Planning, laying the foundation and starting a project of this magnitude takes time, 8 months in this case.

## Conclusions

Our data shows that educating the nurses about the program greatly aided in improving knowledge and achieving buy-in, as evidenced objectively by the pre and post test questionnaires and compliance rates. Constant reinforcement of the technique and knowledge through consistent checks of performance, 6 monthly audits, making training part of nursing competency and most importantly giving feedback of results to stakeholders have enabled us to continue to maintain a high degree of compliance of 98.7%. Future direction of this program includes cost surveillance, introduction of CCHD screening using pulse oximetry to infants being discharged from the NICU making it part of the discharge check list.

## References


1 Hoffman JL, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol. 2002;39(12):1890-1900.

2 Reller MD, Strickland MJ, Riehle-Colarusso T, Mahle WT, Correa A. Prevalence of congenital heart defects in Atlanta, 1998-2005. J Pediatrics 2008;153:807-813.

3 Mahle WT, Newburger JW, Matherne GP, et al; Role of pulse oximetry in examining newborns for congenital heart disease: a scientific statement from the American Heart Association and American Academy of Pediatrics. Circulation. 2009;120(5):447-458.

4 Broussard CS, Gilboa SM, Lee KA, Oster M, Petrini JR, Honein MA. Racial/Ethnic Differences in Infant Mortality Attributable to Birth Defects by Gestational Age. Pediatrics. 2012; 130:e518-27

5 Thangaratinam S, Daniels J, Ewer AK, Zamora J, Khan KS. Accuracy of pulse oximetry in screening for congenital heart disease in asymptomatic newborns: a systematic review. Arch Dis Child Fetal Neonatal Ed. 2007;92(3):F176-F180.

6 Ewer AK. Review of pulse oximetry screening for critical congenital heart defects in newborn infants. Curr Opin Cardiol. 2013;28(2):92-96.

7 Secretary's Advisory Committee on Heritable Disorders in Newborns and Children. HHS Secretary adopts recommendation to add critical congenital heart disease to the Recommended Uniform Screening Panel. September 21, 2011. Washington, DC: US Department of Health and Human Services; 2011.

8 Mehl AL, Thomson V. Newborn hearing screening: the great omission. Pediatrics. 1998;101(1).

9 Kemper AR, Mahle WT, Martin GR, Cooley WC, Kumar P, Morrow WR, Kelm K, Pearson GD, Glidewell J, Grosse SD, Lloyd-Puryear M, Howell RR. Strategies for Implementing Screening for Critical Congenital Heart Disease. Pediatrics. 2011; 128:e1-e8.

# Trends of Staphylococcus Aureus Bloodstream Infections in a Neonatal Intensive Care Unit From 2000-2009

Olajide Dolapo, Ramasubbareddy Dhanireddy and Ajay J Talati

## Abstract

**Background:** Invasive methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive Staphylococcus aureus (MSSA) infections are major causes of numerous neonatal intensive care unit (NICU) outbreaks. There have been increasing reports of MRSA outbreaks in various neonatal intensive care units (NICUs) over the last decade. Our objective was to review the experience of Staphylococcus aureus sepsis in our NICU in the last decade and describe the trends in the incidence of Staphylococcus aureus blood stream infections from 2000 to 2009.

**Methods:** A retrospective perinatal database review of all neonates admitted to our NICU with blood cultures positive for Staphylococcus aureus from (Jan 1st 2000 to December 31st 2009) was conducted. Infants were identified from the database and data were collected regarding their clinical characteristics and co-morbidities, including shock with sepsis and mortality. Period A represents patients admitted in 2000-2003. Period B represents patients seen in 2004-2009.

**Results:** During the study period, 156/11111 infants were identified with Staphylococcus aureus blood stream infection: 41/4486 (0.91%) infants in Period A and 115/6625 (1.73%) in Period B ($p < 0.0004$). Mean gestation at birth was 26 weeks for infants in both periods. There were more MRSA infections in Period B (24% vs. 55% $p < 0.05$) and they were associated with more severe outcomes. In comparing the cases of MRSA infections observed in the two periods, infants in period B notably had significantly more pneumonia cases (2.4% vs. 27%, $p = 0.0005$) and a significantly higher mortality rate (0% vs. 15.7%, $p = 0.0038$). The incidences of skin and soft tissue infections and of necrotizing enterocolitis were not significantly changed in the two periods.

**Conclusion:** There was an increase in the incidence of Staphylococcus aureus infection among neonates after 2004. Although MSSA continues to be a problem in the NICU, MRSA infections were more prevalent in the past 6 years in our NICU. Increased severity of staphylococcal infections and associated rising mortality are possibly related to the increasing MRSA infections with a more virulent community-associated strain.

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## Background

Treatment of Staphylococcus aureus infections in the neonatal intensive care unit (NICU) continues to be a high priority, and reducing the burden of all staphylococcal infections remains of utmost importance. Invasive methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) Staphylococcus aureus bloodstream infections in the newborn present with a wide range of serious complications. The situation is particularly worse in the preterm infant, where the developmental immaturity of the immune system increases the susceptibility to these infections. Complications may include brain or visceral abscesses, meningitis, orbital cellulitis, osteomyelitis, septic arthritis, endocarditis, pneumatoceles and lung abscesses, septic ileus, septic shock and, not infrequently, death [1-5]. Numerous recent outbreaks in the NICUs have been attributed to strains of MRSA found both in the health care environment and in the community. The emergence of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) strains in NICU outbreaks has been widely documented [2,4,6-9]. Earlier studies have stressed the differences between the two different strains of MRSA originating from the hospital environment and from the community, but there are little data available emphasizing the potential change in the epidemiological trend of Staphylococcus aureus blood stream infections in the NICU, with increasing reports of MRSA outbreaks [1,4,8,10-13].

There were reports by the Center for Disease Control (CDC) in the United States showing a series of outbreaks in the NICU of potential new strains of community-acquired MRSA in and around the year 2004 [1,6]. This was also corroborated by Healy et al. [4] in their study in the same period [4].

This study identifies the unique epidemiological characteristics and trends in the incidence of Staphyloccus aureus blood stream infections in neonates, with a view to developing strategies to further decrease the risks of infection. We reviewed our data from 2000-2009, and divided it into cohorts based on references to the increased incidence of MRSA in 2004 in several NICUs.

## Methods

This was a retrospective study carried out in a level III NICU in Memphis, Tennessee, USA—The Regional Medical Center at Memphis. The study was done in a 70-bed NICU with a median annual admission rate of 1110 (range 1006-1200) admissions per year during the study period (2000-2009). Very low birth weight (VLBW) infant admission rate was about 200 per year. The study was approved by the hospital Institutional Review Board

(Reference: 1101514-XM). The NICU perinatal database was used to create a list of infants hospitalized in the NICU with positive blood culture for Staphylococcus aureus (both MSSA and MRSA) in this period. A chart review of all neonates admitted to the NICU with staphylococcal blood stream infection from January 1st 2000 to December 31st 2009 was done.

Subjects were classified into two groups based on the date of hospital admission using the year 2004 as a reference point, which was the year from which earlier reports of MRSA outbreaks in the NICU were documented.

We compared the demographics, clinical characteristics and outcomes of staphylococcal blood stream infections in the periods before and after reported outbreaks of MRSA in the NICU over the last decade. Period A represents infants admitted from January 1st 2000 to December 31st 2003, and Period B comprises infants admitted from January 1st 2004 to December 31st 2009.

## Study design

Data such as gestational age, birth weight, sex, age at diagnosis with a positive blood culture for S. aureus,duration of hospitalization, mechanical ventilation and therapy for respiratory distress syndrome, and use of invasive procedures (including umbilical catheterizations and central venous catheter placements) were collected for the study. Clinical features including pneumonia, skin and soft tissue infections and complications of infection (such as occurrence of septic shock and mortality) were included in the data.

Staphylococcus aureus infection or colonization of other body sites, such as skin, anterior nares, conjunctiva, etc., without concomitant positive bloodstream cultures were excluded from the study.

Data regarding antibiotic susceptibility patterns were collected for the following antibiotics—penicillin, oxacillin, vancomycin and clindamycin. Inducible resistance to clindamycin by the D-zone test was performed on isolates with erythromycin resistance and clindamycin susceptibility. Isolates were categorized into susceptible and resistant groups.

## Definitions of variables

The diagnosis of pneumonia was considered if clinical criteria were met (acute clinical deterioration, pulse oximetry, increased respiratory support requirement), radiological findings (presence of new or changing infiltrate on chest radiography) and laboratory parameters (elevated C-reactive protein or abnormal white cell count) suggestive of bacterial infection.

Necrotizing enterocolitis (NEC) was only considered if there were features of stage II NEC or higher, based on modified Bell's criteria [14].

Skin or soft tissue infections were identified based on the

**Table 1 Characteristics of infants with *Staphylococcus aureus* infection during the two study periods**

| | Period A (n = 41) | | Period B (n = 115) | |
|---|---|---|---|---|
| **Characteristics** | **MSSA (n = 31)** | **MRSA (n = 10)** | **MSSA (n = 51)** | **MRSA (n = 64)** |
| Birth weight (g) | | | | |
| Median ($25^{th}$-$75^{th}$ percentile) | 752 (553-977) | 737 (563-1120) | 838 (647-1081) | 736 (580-945) |
| Category, n (%) | | | | |
| <750 | 16 (52) | 4 (40) | 21 (42) | 37 (58) |
| 751 – 1000 | 7 (23) | 0 (0) | 14 (28) | 17 (26) |
| 1001 – 1250 | 2 (6) | 2 (20) | 8 (16) | 4 (6) |
| 1251 – 1500 | 2 (6) | 2 (20) | 2 (4) | 1 (2) |
| >1500 | 4 (13) | 2 (20) | 5 (10) | 5 (8) |
| Gestational age (weeks) | | | | |
| Median ($25^{th}$-$75^{th}$ percentile) | 27 (25-29) | 27 (24-31) | 27 (26-30) | 27 (25-29) |
| Category, n (%) | | | | |
| 23-25 | 9 (29) | 3 (30) | 9 (18) | 20 (31) |
| 26-28 | 12 (39) | 3 (30) | 25 (48) | 23 (36) |
| 29-31 | 6 (19) | 2 (20) | 11 (22) | 13 (20) |
| ≥32 | 4 (13) | 2 (20) | 6 (12) | 8 (13) |
| Gender | | | | |
| Male (%) | 12 (39) | 5 (50) | 26 (51) | 32 (50) |
| Female (%) | 17 (61) | 5 (50) | 25 (49) | 32 (50) |
| Frequency of invasive procedures | | | | |
| n (%) | 27 (87) | 9 (90) | 46 (90) | 55 (86) |
| Mechanical ventilation | | | | |
| n (%) | 29 (94) | 9 (90) | 47 (92) | 60 (94) |
| Age at diagnosis (days) | | | | |
| Median | 27 | 25 | 22 | 25 |

**Table 2 Survival rates among infants during the two study periods**

| Birth weight distribution (grams) | Period A (N = 41) | | Period B (N = 115) | |
|---|---|---|---|---|
| | MSSA (n = 31) | MRSA (n = 10) | MSSA (n = 51) | MRSA (n = 64)* |
| <750 | 16 | 4 | 21 | 37 |
| Survived (%) | 12 (75) | 4 (100) | 17 (81) | 26 (70) |
| 750 – 999 | 7 | 0 | 14 | 17 |
| Survived (%) | 7 (100) | 0 | 12 (86) | 12 (71) |
| 1000 – 1499 | 2 | 2 | 8 | 4 |
| Survived (%) | 2 (100) | 2 (100) | 8 (100) | 3 (75) |
| 1500 - 1999 | 2 | 2 | 2 | 1 |
| Survived (%) | 2 (100) | 2 (100) | 2 (100) | 0 (0) |
| ≥ 2000 | 4 | 2 | 5 | 5 |
| Survived (%) | 4 (100) | 2 (100) | 5 (100) | (100) |

*Trend analysis of survival rates for MRSA and MSSA infections in the weight categories, using the extended Mantel-Haenszel chi-square for linear trend, showed a significant risk of death when weight was <750 grams for MSSA cases ($p = 0.0166$), but no significant survival trend with increasing gestational age seen in MRSA cases.

individual clinical team's evaluation and diagnosis. Septic shock was defined as the occurrence of hypotension with evidence of sepsis in the presence of a positive blood culture, with or without signs of end-organ dysfunction. It was also identified as shock occurring within 48 hours of positive blood culture. Mortality related to sepsis was considered if it occurred within 14 days of positive culture. Infection rates were expressed as the number of infants infected per 1000 NICU admissions.

Statistical analyses were carried out using chi squared tests to compare categorical variables between groups and the extended Mantel-Haenszel chi squared test for linear trend [15] was used to analyze the trend data. Continuous variables were compared using medians of variables and the interquartile range. Statistical significance was set at $p < 0.05$.

## Results

During the study period, 156 (1.4%) of 11,111 NICU infants were identified with Staphylococcus aureus blood stream infection. Period A (Jan 1st 2000-Dec 31st 2003) had 41 (0.91%) cases out of 4,486 total NICU admissions, while Period B (Jan 1st 2004-Dec 31st 2009), had significantly higher number with 115 (1.73%) cases, of a total of 6,625 infants (p=0.004).

In 2007, education on hygiene and hand-washing methods was intensified and the use of vancomycin locks was introduced (later discontinued in 2009). Otherwise, there were no other changes in the care provided in the two study periods. The total length of stay for VLBW infants in our NICU did not seem to change over time and ranged between 48-61 days mean duration, being 54.7 days in 2000 and 61.6 days in 2009.

As shown in Table 1, the median birth weight and gestation of infants in both periods, irrespective of MSSA or MRSA infection, were similar. The frequency of exposure to invasive procedures and devices was also identical in the two periods (87.8% vs 87.8%) p = 1.000. Mean duration of umbilical catheter days was similar (7.89 ± 6.62 days vs. 7.10 ± 7.23 days) p = 0.543. There was no significant difference in the mechanical ventilation requirements of cohorts in both periods (92.7% vs. 93.0%) p = 1.000. Table 2 shows the sepsis-related mortality in different birth weight groups with both MRSA and MSSA infections. The risk for mortality does not decrease with increasing birth weight with MRSA infections (p = 0.16) as compared to MSSA, where mortality was significantly lower with increasing birth weight ($p < 0.05$).

MRSA infections were significantly higher in Period B (24% vs. 55%, $p < 0.05$) and, as shown in Table 3, were also associated with more severe outcomes. In comparing the cases of MRSA infections observed in these two periods, infants in period B notably had a significantly higher incidence of pneumonia (2.4% vs. 27%, p = 0.0005) and a significantly higher mortality rate (0% vs. 15.7%, p = 0.0038). The incidences of skin and soft tissue infections and that of necrotizing enterocolitis were not significantly different in the two periods. Period B was associated with an increasing trend of septic shock complications, although this was not statistically different from Period A.

**Table 3 Complications of *Staphylococcus aureus* blood stream infections**

| | Period A (2000-2003) n = 41 | | Period B (2004-2009) n = 115 | | *p* value *(comparing MRSA infection in the two periods)* |
|---|---|---|---|---|---|
| *Complications* | MSSA (n = 31) | MRSA (n = 10) | MSSA (n = 51) | MRSA (n = 64) | |
| Septic shock | 0 (0%) | 1 (2.4%) | 4 (3.5%) | 13 (11.3%) | 0.115 |
| Concomitant soft tissue/skin infection | 4 (9.8%) | 6 (14.6%) | 5 (4.3%) | 13 (11.3%) | 0.584 |
| Pneumonia | 6 (14.6%) | 1 (2.4%) | 7 (6.1%) | 31 (27.0%) | 0.0005 |
| Necrotizing enterocolitis | 1 (2.4%) | 4 (9.8%) | 3 (2.6%) | 5 (4.3%) | 0.2435 |
| Mortality | 4 (9.8%) | 0 (0%) | 6 (5.2%) | 18 (15.7%) | 0.0038 |
| Length of hospitalization (mean number of days) | 87.4 ± 40.6 | 80.6 ± 42.4 | 55.7 ± 30.2 | 53.6 ± 36.6 | 0.0371 |

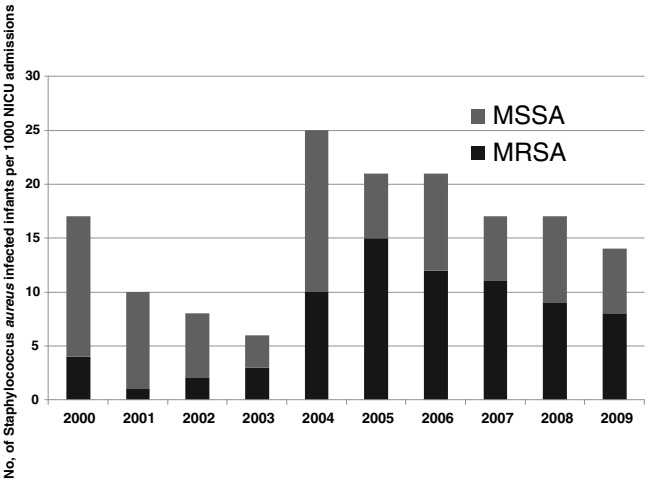


**Figure 1** The yearly trend of MSSA and MRSA infection in the last decade, showing a significant rise in overall incidence of Staphylococcus aureus infections in 2004. The extended Mantel-Haenszel chi-square test for linear trend also showed a significant increase in MRSA infections over the 10-year period (p = 0.0007), but no trend in increase of MSSA infections (p = 0.229).

MRSA-infected infants in period B had a significantly shorter mean length of hospitalization than similarly infected infants in period A (80.6 ± 42.39 vs. 53.6 ± 36.6 total hospital days; p = 0.0371). Infants with MSSA were also noted to have a much shorter hospital course in Period B (87.4 ± 40.6 vs. 55.7 ± 30.2 days; p = 0.0001).

The yearly trend of MRSA versus MSSA infections, with the number of infected infants per 1000 NICU admissions, is shown in the Figure 1. This shows an overall rise in the incidence of Staphylococcus aureus blood stream infections from the year 2004 in our NICU. Analyses of the trend data for MSSA and MRSA infections over the study period were performed using the extended Mantel-Haenszel chi-squared test for linear trend. Results demonstrated a significant increase in trend for MRSA infections, but not for MSSA infections. (MRSA trend analysis p = 0.000702 vs. MSSA p =0.229).

All Staphylococcus aureus isolates (MSSA and MRSA) were susceptible to vancomycin. The sensitivity pattern of MRSA to clindamycin was similar in the two periods: 60% of MRSA isolates were sensitive to clindamycin in Period A vs. 64% in Period B.

## Discussion

According to a 2011 CDC report, the incidence of MRSA in the community in general has increased rapidly in the past decade, with little or no evidence of recent decline, despite clear evidence that invasive MRSA infections in the health care setting is declining [6]. The implementation of aggressive infection control techniques in the health care environment has proved successful in reducing the incidence of health care-associated infections in various NICUs [8].

Our study demonstrates a rise in the overall incidence of Staphylococcus aureus blood stream infections observed in the NICU in the last 10 years, with a peak period around the year 2004. This period coincides with widespread reports of CA-MRSA outbreaks in the NICUs [1,2,4,5,8].

The incidence of MRSA infections in the NICU is still unacceptably high, and this may be likely linked to the acquisition of CA-MRSA strains, which have evolved in the community and penetrated the NICU through either parents or care providers of the patient [8,9,16-19].

During the study period we detected that significantly more MRSA infections were seen in the last 6 years, and that these cases were more frequently associated with severe clinical presentations and worse outcomes. In previous studies, there was earlier onset of MRSA infections compared to MSSA infections, which was attributed to possible vertical transmission of infection [16].

This was not, however, the finding in our study, where the median age at presentation for MSSA infections was 27 days in Period A and 22 days in Period B, while the median age at diagnosis for MRSA infections was 25 days in the two periods.

The rate of skin and soft tissue infections was not significantly different for either MRSA or MSSA cases during the two periods reviewed in the study. Similar findings were reported by Carey et al., who compared 123 infections caused by MSSA and 49 caused by MRSA in a neonatal ICU. The rate of skin and soft tissue infections was similar for both groups at 45% [16]. However, in our study, there may have been an underestimation of skin and soft tissue infection cases, as those without positive blood culture were excluded from the study.

Duration of hospital stay in the second period of our study was significantly less than in the initial 4-year period. It is unclear whether the increased incidence of MRSA infections with more severe complications in the subsequent 6 years led to increased mortality or whether an improvement in neonatal care and management approach led to shorter hospital stay for ELBW infants. An earlier study by Burke et al. found 164 episodes of S. aureus bacteremia in 151 children and infants [3]. In this study, children with MRSA infection stayed in the hospital longer (with a mean of 36 days) than did children with MSSA infection (mean 16.3 days). However, the study was done in a cluster of not just neonates, but children and infants.

In our study, the predominant weight category of all infants with Staphylococcus aureus blood stream infection in the NICU was noted to be less than 750 g (51% of all cases), and they were extremely preterm. Reasons for this were described by Healy et al. [4] in an earlier study, emphasizing risk factors for staphylococcal infections that are peculiar to extremely low birth weight infants; namely, poorly developed host defense mechanisms, central venous catheter requirements, need for endotracheal or upper gastrointestinal tube placement, and procedures that might compromise skin integrity. However, a trend analysis of mortality revealed no change in risk with increasing birth weight in MRSA infections. The risk of death was significantly higher in infants < 750 g birth weight, with MSSA infections. Shane et al. [20] study, demonstrated no significant difference in morbidity or mortality of very low birth weight (VLBW) infants with MRSA compared with those with MSSA bacteremia. This conclusion probably reflected the multi-center nature of their study, as 40% (8 out of 20) of the study centers, actually reported zero cases of MRSA infection. This also probably demonstrated the variability in the population and practice of these study centers.

The exposure of all infected infants in our study, to risk factors was assessed (such as device utilization and exposure to invasive procedures) and no difference was found between study periods in the degree of exposure to risk factors.

Emphasis remains on infection control practices and the prevention of transmission in identified cases. The importance of judicious compliance to standard infection control practices such as hand hygiene, gloving, protection of eyes, nose and mouth; gowning and appropriate handling of patient care equipment and devices cannot be over-emphasized. Contact precautions must be adhered to in all identified cases [6,7].

Our study emphasizes the changing pattern of S. aureus infection in our NICU in the last decade as it relates to increasing reports of MRSA outbreaks. This study is, however, limited by the inability to determine the pathological characteristics and phage-type of isolates, as data were retrospectively collected. The retrospective nature of data collection inherently led to some diagnostic biases. Other limitations that are commonly associated with retrospective chart reviews, such as incomplete documentation, missing data and problematic verification of information are also possible with this study.

## Conclusions

In conclusion, there was an increase in the incidence of S.aureus blood stream infections among neonates after 2003, which coincides with increasing reports of MRSA infections in the NICU. Though, MSSA continues to be a problem in the NICU, MRSA infections were more prevalent in the last 6 years. The increased severity of S aureus infection and associated rising mortality rate may be related to increasing MRSA infections with a more virulent community-associated strain.

## References


1 Centers for Disease Control and Prevention: Outbreaks of community-associated methicillin-resistant Staphylococcus aureus skin infections—Los Angeles County, California, 2002-2003. MMWR Morb Mortal Wkly Rep 2003, 52(5):88.

2 Centers for Disease Control and Prevention: Community-associated methicillin-resistant Staphylococcus aureus infection among healthy newborns—Chicago and Los Angeles County, 2004. MMWR Morb Mortal Wkly Rep 2006, 55(12):329-332.

3 Burke RE, Halpern MS, Baron EJ, Gutierrez K: Pediatric and neonatal Staphylococcus aureus bacteremia: epidemiology, risk factors, and outcome. Infect Control Hosp Epidemiol 2009, 30(7):636-644.

4 Healy CM, Hulten KG, Palazzi DL, Campbell JR, Baker CJ: Emergence of new strains of methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit. Clin Infect Dis 2004, 39(10):1460-1466.

5 Saiman L, Cronquist A, Wu F, Zhou J, Rubenstein D, Eisner W, Kreiswirth BN, Della-Latta P: An outbreak of methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit. Infect Control Hosp Epidemiol 2003, 24(5):317-321.

6 Centers for Disease Control and Prevention: Methicillin-resistant Staphylococcus aureus infections. 2006. http://www.cdc.gov/mrsa/index. html. Accessed 20 June 2012.

7 Abramson MA, Sexton DJ: Nosocomial methicillin-resistant and methicillin-susceptible Staphylococcus aureus primary bacteremia: at what costs? Infect Control Hosp Epidemiol 1999, 20(6):408-411.

8 Chaix C, Durand-Zaleski I, Alberti C, Brun-Buisson C: Control of endemic methicillin-resistant Staphylococcus aureus: a cost-benefit analysis in an intensive care unit. J Am Med Assoc 1999, 282(18):1745-1751.

9 Saiman L, O'Keefe M, Graham PL 3rd, Wu F, Said-Salim B, Kreiswirth B, LaSala A, Schlievert PM, Della-Latta P: Hospital transmission of community-acquired methicillin-resistant Staphylococcus aureus among postpartum women. Clin Infect Dis 2003, 37(10):1313-1319.

10 Carey AJ, Graham PL, Hyman S, Carp D, Wu F, Della-Latta P: Molecular epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) in a l evel-III-IV neonatal intensive care unit (NICU). San Francisco, CA: Abstract 2857.189 Pediatric Academic Society Annual Meetin; 2006.

11 Friedland IR, du Plessis J, Cilliers A: Cardiac complications in children with Staphylococcus aureus bacteremia. J Pediatr 1995, 127(5):746-748.

12 Korakaki E, Aligizakis A, Manoura A, Hatzidaki E, Saitakis E, Anatoliotaki M, Velivasakis E, Maraki S, Giannakopoulou C: Methicillin-resistant Staphylococcus aureus osteomyelitis and septic arthritis in neonates: diagnosis and management. Jpn J Infect Dis 2007, 60(2-3):129-131.

13 Regev-Yochay G, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, Etienne J, Blech M, Smollen G, Maayan-Metzger A, Leavitt A, Rahav G, Keller N: Methicillin-resistant Staphylococcus aureus in neonatal intensive care unit. Emerg Infect Dis 2005, 11(3):453-456.

14 Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, Brotherton T: Neonatal necrotizing enterocolitis: therapeutic decisions based upon clinical staging. Ann Surg 1978, 187:1-7.

15 Rosner B: Fundamentals of Biostatistics. 5th edition. Pacific Grove CA: Duxbury Press; 2000.

16 Carey AJ, Duchon J, Della-Latta P, Saiman L: The epidemiology of methicillin-susceptible and methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit, 2000-2007. J Perinatol 2010, 30(2):135-139.

17 Jahamy H, Ganga R, Al Raiy B, Shemes S, Nagappan V, Sharma M, Riederer K, Khatib R: Staphylococcus aureus skin/soft-tissue infections: the impact of SCCmec type and Panton-Valentine leukocidin. Scand J Infect Dis 2008, 40(8):601-606.

18 McAdams RM, Ellis MW, Trevino S, Rajnik M: Spread of methicillin-resistant Staphylococcus aureus USA300 in a neonatal intensive care unit. Pediatr Int 2008, 50(6):810-815.

19 Saiman L: Preventing infections in the neonatal intensive care unit. In Prevention and Control of Nosocomial Infections. 4th edition. Edited by Wenzel R. Philadelphia, PA: Lippincott, Williams and Wilkins; 2003:342-368.

20 Shane AL, Hansen NI, Stoll BJ, Bell EF, Sánchez PJ, Shankaran S, Laptook AR, Das A, Walsh MC, Hale EC, Newman NS, Schrag SJ, Higgins RD, Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network: Methicillin-resistant and susceptible Staphylococcus aureus bacteremia and meningitis in preterm infants. Pediatrics 2012, 129(4):e914-e922.

# Intestinal Carriage of Methicillin-Resistant Staphylococcus Aureus in Nasal MRSA Carriers Hospitalized in the Neonatal Intensive Care Unit

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## Abstract

**Background:** The current data regarding the correlation between the methicillin-resistant Staphylococcus aureus (MRSA) clones carried in the nasal cavity and digestive tract are inadequate.

**Methods:** MRSA strains were isolated from both the feces and nasal swabs of 21 nasal-MRSA carriers ranging from 10 to 104 days of age treated at the neonatal intensive care units of two hospitals. The molecular epidemiological characteristics of the isolates were determined: multilocus sequence types, spa-types, staphylococcal cassette chromosome mec (SCCmec) types, carriage of four exotoxin genes, and genes contained in commercially available kit.

**Results:** The feces of all nasal carriers contained MRSA at levels ranging from $4.0 \times 10^2$ to $2.8 \times 10^8$ colony forming units/g feces. The MRSA clones isolated from the feces and the nasal swabs of each patient were the same. Four MRSA clones, clonal complex (CC) 8-SCCmec IVl, CC8-SCCmec IVb, CC1-SCCmec IVa and CC5-SCCmec IIa were identified from 21 patients. All CC8-SCCmec IVl strains and one of three CC5-SCCmec IIa strains carried the toxic shock syndrome toxin gene.

**Conclusions:** The feces of tested MRSA carriers contained the same MRSA clones as the nasal isolates in considerable amounts, suggesting that more careful attention should be paid for the handling of excrement in the case of newborn babies or infants than that of adults.

## Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is an important causative pathogen of healthcare-associated infections. It is well known that MRSA strains carried by an infected individuals, asymptomatic carrier or contaminated objects are transmitted via several routes, e.g., direct contact with an infected individual, asymptomatic carrier or contaminated object, the airborne transmission of floating cells, etc. [1]. To control the infection of MRSA, screening of MRSA nasal-carrier is conducted generally at hospitals, since the mucosal membrane of the nasal cavity is a well-known niche for Staphylococcal strains and nasal colonization by MRSA is a well-established risk factor for hospital-acquired MRSA infection among the causes of nosocomial MRSA infection [2-6].

However, MRSA strains colonize at the area other than nasal swabs, and the colonization at those areas was regarded to be another risk factor for MRSA dissemination, too. Universal screening of all hospitalized patients and selective screening limited to high-risk patients, such as those admitted to the intensive care unit or scheduled for surgery, are routinely conducted [7]. About 40% of individuals with nasal colonization are also colonized in other areas, including the throat, perineum and axilla in adults [8,9]. Acton et al. reviewed cases involving the intestinal carriage of S. aureus [10]. Ammeriaan et al. reported that one of the causes of treatment failure for MRSA carriers might be due to the presence of strains colonized at extra-nasal sites [11]. Although MRSA infection is decreasing as results of infection control e.g., performing standard precaution, active surveillance culture, and cohorting, outbreak of MRSA strains still occurred. It has been reported that invasive nosocomial infections predominantly occur in children younger than 1 year of age, with an incidence of 14.7 per 100,000, versus 0.3-1.0 per 100,000 in older children [5] and the risk factors associated with an increased rate of infections in the neonatal intensive care unit (NICU) were suggested. These included the presence of invasive devices, exposure to broad-spectrum antibiotic agents, the use of parenteral nutrition, overcrowding and poor staffing ratios [12].

We presumed that the feces of neonates and infants might contain MRSA strains at considerable amount and would have the possibility to serve as a potential source of MRSA dissemination in the NICU if the contact precautions are inconsistent. Furthermore, the reports comparing the characteristics of MRSA strains isolated from the stool and the nasal cavity in MRSA-positive newborn babies and infants are inadequate. We aimed at isolating and characterizing MRSA strains from the feces of MRSA nasal carriers admitted to the NICU. In this study, we estimated the number of MRSA strains in feces and compared the genetic characteristics of strains isolated from nasal swabs and feces.

## Materials and methods

### Subjects

Patients who were judged to be positive for MRSA by nasal screening were selected from among neonate and infant patients admitted to the NICUs between January 2013 and June 2013 at two university-affiliated tertiary hospitals (J:

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**Table 1 Isolation of MRSA strains from the nasal swabs and feces of the patients in the NICU**

| Patients | Growth on agar plates with antibiotics | | | | Numbers of yellowish colony isolated from stool sample[a] (CFU/g) |
|---|---|---|---|---|---|
| | Nasal swabs | | Feces | | |
| | OXA | CFX | OXA | CFX | |
| A | + | + | + | - | $4.0 \times 10^6$ |
| B | + | + | + | + | $1.6 \times 10^5$ |
| C | + | + | + | + | $2.9 \times 10^6$ |
| D | + | + | + | + | $3.1 \times 10^6$ |
| E | - | + | + | + | $1.6 \times 10^3$ |
| F | + | + | + | + | $2.4 \times 10^6$ |
| G | + | + | + | + | $2.4 \times 10^7$ |
| H | + | + | + | + | $4.7 \times 10^6$ |
| I | + | + | + | - | $6.0 \times 10^4$ |
| J | + | + | + | - | $1.6 \times 10^3$ |
| K | + | + | + | - | $1.1 \times 10^7$ |
| L | + | NT | + | NT | $6.0 \times 10^6$ |
| M | + | + | + | - | $1.6 \times 10^5$ |
| N | + | + | + | + | $2.9 \times 10^6$ |
| O | + | + | + | + | $2.5 \times 10^6$ |
| P | + | + | + | + | $2.8 \times 10^8$ |
| Q | + | + | + | + | $4.0 \times 10^2$ |
| R | + | + | + | + | $1.6 \times 10^6$ |
| S | + | + | + | + | $1.0 \times 10^3$ |
| T | + | + | + | + | $2.7 \times 10^6$ |
| U | + | + | + | + | $2.9 \times 10^6$ |

*Abbreviations*: *OXA* Oxacillin, *CFX* Cefoxitin, *CFU* colony forming units, *NT* not tested.
[a]Average number of yellow-colored colonies grown on agar plates with oxacillin.

Juntendo University Hospital and S:Juntendo University Shizuoka Hospital). At the two hospitals, screening for MRSA was performed on every patient at admission, as well as every two weeks after hospitalization. MRSA screening was conducted by inoculating nasal swabs to CHROMagar MRSA (Kanto Chemical Co., Tokyo, Japan), a selective choromogenic agar containing cephamycin [13]. Briefly, one hundred and sixty-nine patients underwent nasal screening, including 100 males and 69 females ranging from 0 to 171 days of age, with an average age of 19.6 days. The average weight was 2,446 g, ranging from 576 to 4,481 g. A total of 26 of the 169 tested patients were found to be MRSA carriers based on the screening specimens. Among the 26 MRSA carriers, 21 patients were selected for this study. Five patients were excluded; two patients were excluded because they had been administered vancomycin intravenously before sample collection for this study, and three patients were excluded because their feces could not be collected, e.g., they left the hospital before sample collection. The subjects consisted of 11 males and 10 females ranging from 10 to 104 days of age, with an average age of 35.3 days. The average weight was 2,458 g, ranging from 1,186 to 4,545 g. Of the 21 subjects, 18 acquired MRSA during their stay in the hospitals and three were positive on admission. No subjects had gastrointestinal symptoms, such as diarrhea or vomiting.

### Isolation of MRSA strains

Nasal strains were collected using SEEDSWAB No. 2 (Eiken chemical Co. Ltd, Tokyo, Japan) and samples were inoculated onto two separate mannitol salt agar plates, one containing 2 mg/L oxacillin and one 10 mg/L cefoxitin. For fecal samples, 50 mg of stool was diluted with saline to 5% w/v, then was further diluted 102-fold and 104-fold. A 100 Œºl portion of each diluted sample was inoculated on two separate mannitol-salt agars containing 2 mg/L of oxacillin and 10 mg/L of cefoxitin. The number of MRSA strains in each fecal sample was estimated by counting the number of yellow-colored colonies grown on the selective medium. The yellow-colored colonies grown on the plates after incubation at 37 degrees Celsius for 48 hours were regarded to be MRSA strains, which were later confirmed using PCR of mecA and femA.

### Validation and characterization of MRSA strains

We extracted chromosomal DNA from 1-4 yellow-colored colonies on the agar plates using Cica Geneous DNA Extraction Reagent (Kanto chemical Co., Tokyo, Japan) and conducted multiplex PCR using the Cica Geneus Staph POT KIT (Kanto chemical Co., Tokyo, Japan). The kit contained 23 primer pairs identifying femA as a marker of S. aureus, five genes related to Staphylococcal cassette chromosome mec (SCCmec) elements, two genes located on the S. aureus chromosome and 15 genes located on mobile genetic elements, e.g., bacteriophages. The strains were regarded to be MRSA when both mecA and femA were identified.

### Molecular characterization of MRSA

Based on the results of multiplex PCRs with Staph POT kit, representative isolates from the nasal swabs and feces were chosen and characterized according to the multilocus sequence type (MLST), spa type, SCCmec type and presence of exotoxin genes. Chromosomal DNA was extracted using the DNeasy Tissue Kit (Qiagen, Valencia, USA). The SCCmec elements were identified using multiplex PCRs, as described by Kondo et al. [14]. The subtype of each SCCmec type was determined using PCR with primer pairs, as previously described. MLST and spa-type were determined as previously described [15,16]. Carriage of exotoxin genes, including eta and etb for exfoliative toxins a and b, lukS-PV and lukF-PV for Panton-Valentine Leukocidin and tst for Toxic shock syndrome toxin-1 (tst), was detected using PCR, as previously described [17].

### Ethical approval

This study was approved by the Ethical Committee of two participating hospitals and the written informed consent was obtained from the person in parental authority for the collection of samples and the publication of the analysed results.

## Results

### Isolation of MRSA strains from feces and nasal swabs

The feces of 21 MRSA screening-positive patients was diluted and inoculated on two separate mannitol-salt agar plates containing oxacillin or cefoxitin. Many yellow-colored colonies grew on the agar plates in all patients (Table 1). The number of yellow-colored colonies ranged from $4.0 \times 10^2$ to $2.8 \times 10^8$ of colony forming units (CFU)/ g feces, with $1.7 \times 10^7$ CFU/g feces on average. At the same time, the nasal swabs of 21 patients were streaked onto two separate mannitol-salt agar plates containing oxacillin or cefoxitin. Many yellow-colored colonies grew on the plates in all patients. One to four yellow-colored isolates from the nasal and fecal samples were chosen at random and subjected to multiple PCR with the Staph POT KIT. All tested strains were mecA-and femA-positive and therefore classified as MRSA. The data indicated that the feces of the patients with nasal colonization contained MRSA strains at significant amounts, although the numbers of MRSA colonies varied.

**Table 2 Characterization of the isolates using multiple PCR**

| Patients | Samples | Number of tested strains | Numbers of amplified DNA fragments[a] with relate to | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | ORFs[b] on SCC*mec* elements | | ORFs[c] on chromosome | | ORFs[d] on mobile genetic elements | |
| A | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV1998 | 4 / 4 |
| | Feces | 2 | IS*1272* / type 2 *ccr A* | 2 / 2 | MW0919 | 2 / 2 | SA1774 / SaGlm / SAV1998 | 2 / 2 |
| B | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| C | Nasal swab | 4 | *mecI* / type 2 *ccrA /kdpC* | 4 / 4 | SA2259 | 4 / 4 | *tnpB* / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SAV0913 / SLTorf182 / PV83orf2 | 4 / 4 |
| | Feces | 4 | *mecI* / type 2 *ccrA /kdpC* | 4 / 4 | SA2259 | 4 / 4 | *tnpB* / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SAV0913 / SLTorf182 / PV83orf2 | 4 / 4 |
| D | Nasal swab | 4 | *mecI* / type 2 *ccrA /kdpC* | 4 / 4 | SA2259 | 4 / 4 | *tnpB* / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SAV0913 / SLTorf182 / PV83orf2 | 4 / 4 |
| | Feces | 4 | *mecI* / type 2 *ccrA /kdpC* | 4 / 4 | SA2259 | 4 / 4 | *tnpB* / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SAV0913 / SLTorf182 / PV83orf2 | 4 / 4 |
| E | Nasal swab | 2 | IS*1272* / type 2 *ccr A* | 2 / 2 | MW0919 | 2 / 2 | SA1774 / SaGlm / SAV0881 / SA1801 | 2 / 2 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| F | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / PV83orf2 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / PV83orf2 | 4 / 4 |
| G | Nasal swab | 3 | IS*1272* / type 2 *ccr A* | 3 / 3 | MW0919 | 3 / 3 | *tnpB* / SaGlm / SLTorf182 / PV83orf2 | 2 / 3 |
| | | | | | | | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / PV83orf2 | 1 / 3 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SaGlm / SLTorf182 / PV83orf2 | 4 / 4 |
| H | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SLTorf182 / PV83orf2 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SLTorf182 / PV83orf2 | 2 / 4 |
| | | | | | | | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SLTorf182 / PV83orf2 | 2 / 4 |
| I | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SaGlm / SLTorf182 / PV83orf2 | 4 / 4 |
| | Feces | 2 | IS*1272* / type 2 *ccr A* | 2 / 2 | MW0919 | 2 / 2 | *tnpB* / SaGlm / SLTorf182 / PV83orf2 | 2 / 2 |
| J | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| | Feces | 2 | IS*1272* / type 2 *ccr A* | 2 / 2 | MW0919 | 2 / 2 | SA1774 / SaGlm / SAV0881 / SA1801 | 2 / 2 |
| K | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SLTorf182 / PV83orf2 | 3 / 4 |
| | | | | | | | *tnpB* / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SLTorf182 / PV83orf2 | 1 / 4 |
| | Feces | 2 | IS*1272* / type 2 *ccr A* | 2 / 2 | MW0919 | 2 / 2 | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / SLTorf182 / PV83orf2 | 2 / 2 |

## Comparisons of the carriage of ORFs by fecal and nasal strains

To compare MRSA strains isolated from feces and nasal swabs of each patient, we firstly conducted two multiplex PCRs with Staph POT KIT by choosing 1-4 strains obtained from the feces and nasal swabs of the 21 patients. Two multiplex PCRs could amplify DNA fragments with related to SCCmec elements, two open reading frames (ORF) on the chromosome and 15 ORFs on the mobile genetic elements, e.g., lysogenized bacteriophages as listed in Table 2. Representative banding patterns of amplified DNA fragments from nasal and fecal strains isolated from the same patientare shown in Figure1. In 17 of the 21 patients, exactly the same size and number of DNA fragments were generated using DNA samples from the feces and nasal swabs. In three patients (G, H and K), the dominant strains were identical, but other strains exhibiting different amplification patterns with ORFs in lysogenized bacteriophages were also identified: a nasal isolate of patient G, a fecal isolate of patient H and a nasal isolate of patient K. In a patient (L), different ORFs related to lysogenized bacteriophages were generated, although the amplified DNA fragments in association with the five ORFs in the SCCmec elements and two ORFs on the chromosome were the same.

## Molecular characterization of the MRSA strains

The MLST genotypes, SCCmec types and spa types were determined in one representative isolate from each feces sample among the 21 patients. Three clonal complexes (CC1, CC5 and CC8), four SCCmec types (IIa, IVa, IVb and IVl), and six spa types (2, 59, 606, 855, 1499 and 1500) were identified. Consequently, 21 strains were classified into four clones (Table 3). CC8-SCCmec IVl was the most prominent clone (10 of 21), followed by CC1-SCCmec IVa (7 of 21), CC5-SCCmec IIa (3 of 21) and CC8-SCCmec IVb (1 of 21). The carriage of the four exotoxin genes was examined. Eleven of the 21 strains carried

**Table 2 Characterization of the isolates using multiple PCR** *(Continued)*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| L | Nasal swab | 1 | IS*1272* / type 2 *ccr A* | 1 / 1 | MW0919 | 1 / 1 | *tnpB*/ SAV1774 / SaGlm / SAV0881 / SAV1801 | 1 / 1 |
| | Feces | 1 | IS*1272* / type 2 *ccr A* | 1 / 1 | MW0919 | 1 / 1 | SAV0886 / SA1774 / SAV0885 / SaGlm / SAV0881 / SAV1801 | 1 / 1 |
| M | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / PV83orf2 | 4 / 4 |
| | Feces | 2 | IS*1272* / type 2 *ccr A* | 2 / 2 | MW0919 | 2 / 2 | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / PV83orf2 | 2 / 2 |
| N | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / PV83orf2 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | *tnpB* / SAV0850 / SAV0898 / SAV0866 / SAV1974 / SAV0855 / SaGlm / SLTorf175 / PV83orf2 | 4 / 4 |
| O | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| P | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| Q | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| R | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SAV0898 / SAV1774 / SAV0855 / SaGlm / SLTorf175 / SAV0913 / SAV1998 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SAV0898 / SAV1774 / SAV0855 / SaGlm / SLTorf175 / SAV0913 / SAV1998 | 4 / 4 |
| S | Nasal swab | 4 | *mecI* / type 2 *ccrA /kdpC* | 4 / 4 | SA2259 | 4 / 4 | *tnpB* / SAV0898 / SAV0866 / SAV1774 / SAV0855 / SaGlm / SLTorf175 / SAV1801 / SAV0913 / SAV1998 / PV83orf2 | 4 / 4 |
| | Feces | 4 | *mecI* / type 2 *ccrA /kdpC* | 4 / 4 | SA2259 | 4 / 4 | *tnpB* / SAV0898 / SAV0866 / SAV1774 / SAV0855 / SaGlm / SLTorf175 / SAV1801 / SAV0913 / SAV1998 / PV83orf2 | 4 / 4 |
| T | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| U | Nasal swab | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |
| | Feces | 4 | IS*1272* / type 2 *ccr A* | 4 / 4 | MW0919 | 4 / 4 | SA1774 / SaGlm / SAV0881 / SA1801 | 4 / 4 |

*Abbreviations*: *ORFs* open reading frames.
[a]The ORFs amplified by two multiplex PCR using the Cica Geneus Staph POT KIT are listed.
[b]PCR-positive ORFs related to SCCmec elements. The results of PCR of five genes, mecA, mecI, IS1272, type 2 ccrA, and kdpC located in type IIa SCCmec, are listed. PCR-positive genes, except for mecA, are listed.
[c]PCR-positive ORFs located on the chromosomes of N315(SA2259) and MW2(MW0919).
[d]PCR-positive ORFs related to mobile genetic elements are listed. Fifteen ORFs were targeted for PCR, as follows: *tnp*B in the transposon Tn*554*, a ORF on the genome islands SaGlm, and 12 ORFs on the lysogenized bacteriophages (SA1774 and SA1801 for phi N315; SAV0881, SAV0850, SAV0855, SAV0866, SAV0881, SAV0898 and SAV0913 for phi Mu50A; SAV1974 and SAV1998 for phi Mu50B; orf 175 and orf 182 for phi SLT; and orf2 for phi PV83).

the tst gene, including 10 CC8-SCCmec IVl strains and one CC5-SCCmec IIa strain. In contrast, the eta, etb an lukS, F-PV genes were not identified in any of the tested strains. Next, we chose one representative isolate from each nasal swab among the 21 patients and examined the SCCmec types, spa-types and exotoxin repertoire. The characteristics of the nasal strains were exactly identical to those of the fecal strains. Taken together with the results of multiplex PCR shown in Table 2, the feces of the nasal carriers of MRSA contained the same contained MRSA. Adlerberth I. et al. reported the rates of MRSA clones as the strain isolated from the nasal swabs. S. aureus isolation from feces were 40% to 80% in healthy Three MRSA clones, CC5-SCCmec IIa, CC8-SCCmec newborns and infants ranging from 7 days to 1 year of age IVl and CC8-SCCmec IVb, were identified at the J hos-[18,19]. Acton et al. reported a detection rate of intestinal pital and one clone, CC1-SCCmec IVa, was identified at carriage in healthy individuals and patients of 20% for S. the S hospital. aureus and 9% for MRSA, which is approximately half of that observed for nasal carriage [10]. Here we showed that Discussion infant-nasal MRSA carriers consistently evacuate a lot of The feces of the infants contained significant amount of cells of MRSA in their feces. Many reports advocate estab-MRSA pathogens lishing better screening methods for identifying MRSA In this study, we found that the feces of all 21 patients, carriers by evaluating optimum surveillance sites, e.g., the from whom nasal cavity MRSA strains were isolated, nasal cavity, skin, feces and/or rectum, in order to isolate pathogens [8,9]. To screen for MRSA as part of an active surveillance program, nasal swabs are usually used because the method is easy to perform and has higher sensitivity than other methods, while other sites have been used for complementation [2,7,20].

A lot of cells of MRSA were isolated from the feces of infants. The amount was greater than those reported in adults, similar to the previous report [21]. The intestinal flora of adults is generally occupied by established micro-flora, which may help to prevent the colonization of newly incorporated bacteria, known as the phenomenon of 'colonization resistance' [22]. In contrast, the intestinal flora of neonates and infants, especially premature babies admitted to the NICU, have not yet been established. Therefore, we presume that MRSA strains are able to propagate in or colonize the intestinal tract of infants more efficiently than that of adults.

## The feces of nasal MRSA carriers is associated with a high risk for the dissemination of MRSA

In hospitals, the horizontal transmission of infective substances from an infected patient to another patient via contact with medical staff is likely to occur, as previously described. However, transmission via direct contact can be controlled by thoroughly implementing standard precautions, regardless of the presence of MRSA colonization. Furthermore, it is widely recognized that the stool and vomit excreted from gastroenteritis patients contain many pathogens, e.g., rotavirus, which can

**Table 3 Molecular characterization of the MRSA strains isolated from the feces and nasal swabs**

| Strains[a] | MLST | | *spa* types | SCC*mec* types | Exotoxin genes | | | | Isolated at[b] |
|---|---|---|---|---|---|---|---|---|---|
| | ST | CC | | | *tst* | *eta* | *etb* | *lukS, F-PV* | |
| A*f* | 8 | 8 | 1499 | IVl | + | - | - | - | *J* |
| A*n* | NT | NT | 1499 | IVl | + | - | - | - | |
| B*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| B*n* | NT | NT | 606 | IVl | + | - | - | - | |
| C*f* | 764 | 5 | 2 | IIa | - | - | - | - | *J*[c] |
| C*n* | NT | NT | 2 | IIa | - | - | - | - | |
| D*f* | 764 | 5 | 2 | IIa | - | - | - | - | *J*[c] |
| D*n* | NT | NT | 2 | IIa | - | - | - | - | |
| E*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| E*n* | NT | NT | 606 | IVl | + | - | - | - | |
| F*f* | 2763 | 1 | 1500 | IVa | - | - | - | - | *S* |
| F*n* | NT | NT | 1500 | IVa | - | - | - | - | |
| G*f* | 2764 | 1 | 855 | IVa | - | - | - | - | *S* |
| G*n* | NT | NT | 855 | IVa | - | - | - | - | |
| H*f* | 2764 | 1 | 855 | IVa | - | - | - | - | *S* |
| H*n* | NT | NT | 855 | IVa | - | - | - | - | |
| I*f* | 1 | 1 | 855 | IVa | - | - | - | - | *S* |
| I*n* | NT | NT | 855 | IVa | - | - | - | - | |
| J*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| J*n* | NT | NT | 606 | IVl | + | - | - | - | |
| K*f* | 2763 | 1 | 855 | IVa | - | - | - | - | *S* |
| K*n* | NT | NT | 855 | IVa | - | - | - | - | |
| L*f* | 8 | 8 | 59 | IVb | - | - | - | - | *J* |
| L*n* | NT | NT | 59 | IVb | - | - | - | - | |
| M*f* | 2763 | 1 | 855 | IVa | - | - | - | - | *S* |
| M*n* | NT | NT | 855 | IVa | - | - | - | - | |
| N*f* | 2763 | 1 | 855 | IVa | - | - | - | - | *S* |
| N*n* | NT | NT | 855 | IVa | - | - | - | - | |
| O*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| O*n* | NT | NT | 606 | IVl | + | - | - | - | |
| P*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| P*n* | NT | NT | 606 | IVl | + | - | - | - | |
| Q*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| Q*n* | NT | NT | 606 | IVl | + | - | - | - | |
| R*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| R*n* | NT | NT | 606 | IVl | + | - | - | - | |
| S*f* | 5 | 5 | 2 | IIa | + | - | - | - | *J*[c] |
| S*n* | NT | NT | 2 | IIa | + | - | - | - | |
| T*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| T*n* | NT | NT | 606 | IVl | + | - | - | - | |
| U*f* | 8 | 8 | 606 | IVl | + | - | - | - | *J* |
| U*n* | NT | NT | 606 | IVl | + | - | - | - | |

*Abbreviations*: *f* feces, *n* nasal swabs, *NT* not tested.
[a]Strains isolated from feces and nasal swabs of patients A-U.
[b]Strains isolated at two hospitals: Juntendo University Hospital (*J*) and Juntendo University Shizuoka Hospital (*S*).
[c]Patients C, D and S had already been colonized with MRSA before being transferred to hospital J from previous facilities.

cause a secondary infection [23-25]. We suggest that healthcare workers should recognize the feces from nasal MRSA carriers as a potential source of MRSA strains that cause transmission, although gastrointestinal symptoms may not occur in all patients with fecal MRSA colonization [26]. Feces containing MRSA may serve as a source of contamination due to the possibility of spreading to surrounding surfaces by contact with healthcare workers' hands. Our data suggested that more careful attention should be paid for the handling of excrement in the case of newborn babies or infants than that of adults. When changing diapers or cloths and bathing neonates and infants in the NICU, healthcare workers may come in contact with MRSA. In the cases of treatment failure, e.g., inappropriate precautions, hand washing or hand hygiene using antiseptic agents, the transmission of MRSA is likely to occur. Although there are several studies regarding the intestinal carriage of S. aureus including MRSA, there were few studies that examined molecular epidemiological characteristics of MRSA strains from two sources at the same time. In this study, the MRSA clones contained in the feces were identical to those isolated from the nasal cavity, suggesting that MRSA strains in the nasal cavity are carried into the intestines, and the feces of MRSA nasal carriers should be regarded as a source of transmission of MRSA. Since the number of MRSA colonies in the feces was greater than that observed in the nasal cavity, the risk of transmission is higher in cases involving contact with feces than with nasal secretions. However, the subjects of this study were chosen from the patients who admitted to NICU. Threfore, it is unclear whether the same results were obtained in the cases of healthy infants or adult patients. Further research need to be conducted to clarify these questions.

## Characteristics MRSA clones isolated from feces and nasal swabs

To know identities of MRSA clones contained in the feces and nasal swabs, we firstly screened MRSA strains in plural with multiplex PCRs that can identify ORFs in SCCmec elements, some chromosomal genes of CC1 and CC5 strains, and genes in mobile genetic elements, e.g., bacteriophage. We did not regard the method as a one to be used instead of pulsed field gel electrophresis or to be used to determine MRSA clone. Here we used the PCRs as a compendium method to examine the identities of the carriage of tested ORFs between several strains. A majority of strain isolated from feces and nasal swabs of every one person were the same, suggesting that the same clone might be isolated. These data indicated that MRSA strains at the nasal cavity entered to digestive tract, and propagated there.

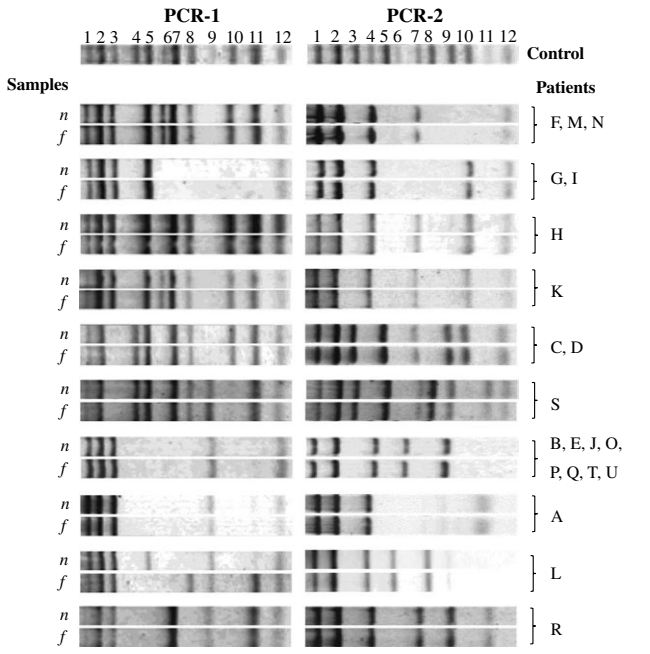


**Figure 1** Comparisons of amplified DNA fragments with two multiplex PCRs. Abbreviations: n, isolates from nasal swabs; f, isolates from feces. For PCR-1: 1, femA; 2, mecA; 3, IS1272; 4, kdpC; 5, tnpB in Tn554; 6, ORF SV0850 in phi mu1; 7, ORF SAV0898 in phi mu1; 8, ORF SAV0866 in phi mu1; 9, ORF SA1774 in phi N315; 10, ORF SAV1974 in phi Sa 3mu; 11, ORF SAV0855 in phi mu1; 12, Genomic Island SaGIm. For PCR-2: 1, femA; 2, type 2 ccrA; 3, SA2259; 4, MW0919; 5, mecI; 6, ORF SAV0881 in phi mu1; 7, ORF 175 in phi SLT; 8, ORF SA1801 in phi N315; 9, ORF SAV0913 in phi mu1; 10, ORF 182 in phi SLT; 11, ORF SAV1998 in phi Sa 3mu; 12, ORF 2 in phi PV83.

Further detailed investigation revealed characteristic of MRSA strains of two hospitals. At the J hospital, the CC8-SCCmec IVl clone was predominant, although two other clones, CC5-SCCmec IIa and CC8-SCCmec IVb, were also identified. At the S hospital, only the CC1-SCCmec IVa clone was identified. Of the 21 carriers, three were positive on admission to the units and 18 acquired MRSA during their stay in hospitals. Three CC5SCCmec IIa strains were identified in the feces of patients transferred from other facilities who carried MRSA before hospitalization in the J hospital. These data suggest that 18 patients acquired the specific MRSA strains disseminated at each hospital. Type II SCCmec strains are still dominant in Japanese hospitals, and there are reports that type IV SCCmec strains are disseminated in the Japanese community [27]. Yanagihara et al. reported that SCCmec IV strains are predominantly distributed in the outpatient clinics [28]. It is curious that the MRSA clones disseminated in the NICU were not identical to dominant MRSA clone disseminating in other wards, but rather were similar to those isolated from the outpatient departments. It is well known that the epidemiological distribution of MRSA strains varies according to region and age [29-32]. MRSA strains are classified into healthcare-associated (HA-MRSA) and community-associated (CA-MRSA) strains [33,34]. Recent studies have shown that MRSA strains identified in the community showed characteristics that were distinct from the HA-MRSA, and such CA-MRSA clones are sometimes isolated from hospitals [30].

Recently, Iwao et al. reported that CC8-SCCmec IVl strains that carry the toxic shock syndrome toxin gene have been detected in the Japanese community [35]. This finding is confirmed by our observation that the CC8-SCCmec IVl strain is the dominant strain isolated from outpatient clinics of dermatology (Hosoya et al. unpublished data). In the current study, SCCmec IV strains were dominantly isolated from patients admitted to our hospital at birth. These data suggest that MRSA clones emerging in the community are transmitted into and disseminated throughout the hospital. Toxic shock syndrome toxin is a well-known super antigen that causes neonatal toxic shock syndrome-like exanthematous disease in neonates and infants [36]. All tst-positive isolates produce a considerable amount of toxic shock syndrome toxins. Fortunately, most patients did not experience severe symptoms, although the possibility of life-threatening syndrome remains. The root of transmission for the transfer of MRSA clones originating in the Japanese community into the NICU remains unknown; however, one possibility is that the pathogen is brought into the hospital by the patient's mother or other family members. Studies investigating the transmission of MRSA clones colonized in the nasal cavity in family members are required to clarify this issue.

## Conclusions

The feces of the investigated MRSA carriers contained the same MRSA clones obtained from the nasal swabs in considerable amounts, suggesting that the feces of MRSA carriers is associated with a high risk for disseminating MRSA.

## References

1 Kaplan SL, Hulten KG, Mason EO: STAPHYLOCOCCUS AUREUS INFECTIONS. In Textbook of Pediatric Infectious Diseases. Volume 1. 7th edition. Edited by Cherry JD, Harrison GJ, Kaplan SL, Steinbach WJ, Hotez PJ. Philadelphia: ELSEVIER SAUNDERS; 2013:1113-1130.

2 Harbarth S, Hawkey PM, Tenover F, Stefani S, Pantosti A, Struelens MJ: Update on screening and clinical diagnosis of meticillin-resistant Staphylococcus aureus (MRSA). Int J Antimicrob Agents 2011, 37(2):110-117.

3 Santos RP, Mayo TW, Siegel JD: Healthcare epidemiology: active surveillance cultures and contact precautions for control of multidrug-resistant organisms: ethical considerations. Clin Infect Dis 2008, 47(1):110-116.

4 von Eiff C, Becker K, Machka K, Stammer H, Peters G: Nasal carriage as a source of Staphylococcus aureus bacteremia. Study Group. N Engl J Med 2001, 344(1):11-16.

5 Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridlin SK: Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA 2007, 298(15):1763-1771.

6 Milstone AM, Goldner BW, Ross T, Shepard JW, Carroll KC, Perl TM: Methicillin-resistant Staphylococcus aureus colonization and risk of subsequent infection in critically ill children: importance of preventing nosocomial methicillin-resistant Staphylococcus aureus transmission. Clin Infect Dis 2011, 53(9):853-859.

7 Tacconelli E, De Angelis G, de Waure C, Cataldo MA, La Torre G, Cauda R: Rapid screening tests for meticillin-resistant Staphylococcus aureus at hospital admission: systematic review and meta-analysis. Lancet Infect Dis 2009, 9(9):546-554.

8 Coello R, Jimenez J, Garcia M, Arroyo P, Minguez D, Fernandez C, Cruzet F, Gaspar C: Prospective study of infection, colonization and carriage of methicillin-resistant Staphylococcus aureus in an outbreak affecting 990 patients. Eur J Clin Microbiol Infect Dis 1994, 13(1):74-81.

9 Eveillard M, de Lassence A, Lancien E, Barnaud G,

Ricard JD, Joly-Guillou ML: Evaluation of a strategy of screening multiple anatomical sites for methicillin-resistant Staphylococcus aureus at admission to a teaching hospital. Infect Control Hosp Epidemiol 2006, 27(2):181-184.
10 Acton DS, Plat-Sinnige MJ, van Wamel W, de Groot N, van Belkum A: Intestinal carriage of Staphylococcus aureus: how does its frequency compare with that of nasal carriage and what is its clinical impact? Eur J Clin Microbiol Infect Dis 2009, 28(2):115-127.
11 Ammerlaan HS, Kluytmans JA, Berkhout H, Buiting A, de Brauwer EI, van den Broek PJ, van Gelderen P, Leenders SA, Ott A, Richter C, Spanjaard L, Spijkerman IJ, van Tiel FH, Voorn GP, Wulf MW, van Zeijl J, Troelstra A, Bonten MJ: Eradication of carriage with methicillin-resistant Staphylococcus aureus: determinants of treatment failure. J Antimicrob Chemother 2011, 66(10):2418-2424.
12 Polin RA, Denson S, Brady MT: Epidemiology and diagnosis of health care-associated infections in the NICU. Pediatrics 2012, 129(4):e1104-e1109.
13 Diederen B, van Duijn I, van Belkum A, Willemse P, van Keulen P, Kluytmans J: Performance of CHROMagar MRSA medium for detection of methicillin-resistant Staphylococcus aureus. J Clin Microbiol 2005, 43(4):1925-1927.
14 Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K: Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr,and major differences in junkyard regions. Antimicrob Agents Chemother 2007, 51(1):264-274.
15 Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG: Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 2000, 38(3):1008-1015.
16 Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN: Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 1999, 37(11):3556-3563.
17 Ma XX, Ito T, Chongtrakool P, Hiramatsu K: Predominance of clones carrying Panton-Valentine leukocidin genes among methicillin-resistant Staphylococcus aureus strains isolated in Japanese hospitals from 1979 to 1985. J Clin Microbiol 2006, 44(12):4515-4527.
18 Lindberg E, Nowrouzian F, Adlerberth I, Wold AE: Long-time persistence of superantigen-producing Staphylococcus aureus strains in the intestinal microflora of healthy infants. Pediatr Res 2000, 48(6):741-747.
19 Adlerberth I, Strachan DP, Matricardi PM, Ahrne S, Orfei L, Aberg N, Perkin MR, Tripodi S, Hesselmar B, Saalman R, Coates AR, Bonanno CL, Panetta V, Wold AE: Gut microbiota and development of atopic eczema in 3 European birth cohorts. J Allergy Clin Immunol 2007, 120(2):343-350.
20 Centers for Disease Control and Prevention: Methicillin-resistant Staphylococcus aureus (MRSA) infections. 2010, [http://www.cdc.gov/hai/ pdfs/toolkits/MRSA_toolkit_ white_020910_v2.pdf]
21 Lindberg E, Adlerberth I, Hesselmar B, Saalman R, Strannegard IL, Aberg N, Wold AE: High rate of transfer of Staphylococcus aureus from parental skin to infant gut flora. J Clin Microbiol 2004, 42(2):530-534.
22 Van der Waaij D, Van der Waaij BD: The colonization resistance of the digestive tract in different animal species and in man; a comparative study. Epidemiol Infect 1990, 105(2):237-243.
23 Curtis V, Cairncross S: Effect of washing hands with soap on diarrhoea risk in the community: a systematic review. Lancet Infect Dis 2003, 3(5):275-281.
24 Lee GM, Salomon JA, Friedman JF, Hibberd PL, Ross-Degnan D, Zasloff E, Bediako S, Goldmann DA: Illness transmission in the home: a possible role for alcohol-based hand gels. Pediatrics 2005, 115(4):852-860.
25 Rodriguez WJ, Kim HW, Brandt CD, Yolken RH, Richard M, Arrobio JO, Schwartz RH, Kapikian AZ, Chanock RM, Parrott RH: Common exposure outbreak of gastroenteritis due to type 2 rotavirus with high secondary attack rate within families. J Infect Dis 1979, 140(3):353-357.
26 Sugiyama Y, Okii K, Murakami Y, Yokoyama T, Takesue Y, Ohge H, Sueda T, Hiyama E: Changes in the agr locus affect enteritis caused by methicillin-resistant Staphylococcus aureus. J Clin Microbiol 2009, 47(5):1528-1535.
27 Hisata K, Kuwahara-Arai K, YamanotoM,Ito T, Nakatomi Y, CuiL,Baba T, Terasawa M, Sotozono C, Kinoshita S, Yamashiro Y, Hiramatsu K: Dissemination of methicillin-resistant staphylococci among healthy Japanese children. J Clin Microbiol 2005, 43(7):3364-3372.
28 Yanagihara K, Araki N, Watanabe S, Kinebuchi T, Kaku M, Maesaki S, Yamaguchi K, Matsumoto T, Mikamo H, Takesue Y, Kadota J, Fujita J, Iwatsuki K, Hino H, Kaneko T, Asagoe K, Ikeda M, Yasuoka A, Kohno S: Antimicrobial susceptibility and molecular characteristics of 857 methicillin-resistant Staphylococcus aureus isolates from 16 medical centers in Japan (2008-2009): nationwide survey of community-acquired and nosocomial MRSA. Diagn Microbiol Infect Dis 2012, 72(3):253-257.
29 Kluytmans-Vandenbergh MF, Kluytmans JA: Community-acquired methicillin-resistant Staphylococcus aureus: current perspectives. Clin Microbiol Infect 2006, 12(Suppl 1):9-15.
30 David MZ, Glikman D, Crawford SE, Peng J, King KJ, Hostetler MA, Boyle-Vavra S, Daum RS: What is community-associated methicillin-resistant Staphylococcus aureus? JInfect Dis 2008, 197(9):1235-1243.
31 Otter JA, French GL: Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Europe. Lancet Infect Dis 2010, 10(4):227-239.
32 Chuang YY, Huang YC: Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Asia. Lancet Infect Dis 2013, 13(8):698-708.
33 Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, Fridkin S, O'Boyle C, Danila RN, Lynfield R: Comparison of community-and health care-associated methicillin-resistant Staphylococcus aureus infection. JAMA 2003, 290(22):2976-2984.
34 Salgado CD, Farr BM, Calfee DP: Community-acquired methicillin-resistant Staphylococcus aureus: a meta-analysis of prevalence and risk factors. Clin Infect Dis 2003, 36(2):131-139.
35 Iwao Y, Takano T, Higuchi W, Yamamoto T: A new staphylococcal cassette chromosome mec IV encoding a novel cell-wall-anchored surface protein in a major ST8 community-acquired methicillin-resistant Staphylococcus aureus clone in Japan. J Infect Chemother 2012, 18(1):96-104.
36 Kikuchi K, Takahashi N, Piao C, Totsuka K, Nishida H, Uchiyama T: Molecular epidemiology of methicillin-resistant Staphylococcus aureus strains causing neonatal toxic shock syndrome-like exanthematous disease in neonatal and perinatal wards. J Clin Microbiol 2003, 41(7):3001-3006.

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| Plastic feeding containers are exposed to heat greater than 150° F to warm and/or thaw feedings | | | Plastic feeding containers are exposed to heat greater than 150° F to warm and/or thaw feedings | ✓ |
| Warmer is able to warm feedings from the refrigerator to feeding temperature | ✓ | | Warmer is able to warm feedings from the refrigerator to feeding temperature | ✓ |
| Silent operation for optimal protection of cognitive development | ✓ | | Silent operation for optimal protection of cognitive development | |
| One step frozen to feeding cycle | ✓ | | One step frozen to feeding cycle | |
| Device gently mixes to keep lipids and fortifiers in solution | ✓ | | Device gently mixes to keep lipids and fortifiers in solution | |
| Device is intuitive and warms based on the milk's starting temperature not based on a countdown system | ✓ | | Device is intuitive and warms based on the milk's starting temperature not based on a countdown system | |
| Thaws in less than 20 minutes | ✓ | | Thaws in less than 20 minutes | |
| Quad device is optimized for use in pods or nutritional preparation areas/rooms | ✓ | | Quad device is optimized for use in pods or nutritional preparation areas/rooms | |
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