ICMR CENTER FOR ADVANCED RESEARCH IN NEWBORN HEALTH

FINAL REPORT

JUNE 2016

Department of Pediatrics

AIIMS, New Delhi

CONTENTS

FOREWORD

We are honored to present the Report of the Centre for Advanced Research in Newborn Health (CAR-NH) at AIIMS, New Delhi, generously supported by the Indian Council of Medical Research.

In this journey, we attempted to focus on some of the key research priorities in newborn health, namely, infections, nutrition and long term outcomes of 'at risk' neonates. We also contributed to developing ethics guidelines for pediatric research and to develop research capacity. This report summarizes our work. A considerable body of work is either published or under publication.

This program is unique in a very important way. We are a 'Collaborative Research Network' rather than a 'Centre'. Four academic institutions joined hands to develop a high quality research program in neonatal sepsis; and two institutions worked on the preterm feeding. We are proud of our esteemed colleagues and collaborators from Maulana Azad Medical College, VMMC and Safdarjang Hospital, and Chacha Nehru Bal Chikitsalaya for their commitment, leadership and scientific contribution. We believe, this model has a great value and potential. No single institution today can address any big research question. Collaboration gives better value for precious resources and makes the new knowledge more generalizable.

Scientific Advisory Committee, a group of most eminent leaders in the field, has been a source of strength and guidance for the team. It provided constructive suggestions and gentle encouragement all through this program. The scientific merit of the program owes a great deal to the insightful ideas of the Committee.

The Council recognized newborn health as a special area to support and gave us an opportunity to implement a multi-faceted research program through a munificent grant. The leadership of the ICMR and the colleagues at the Division of RHN facilitated our efforts in every possible way.

This research program has advanced knowledge in certain areas of newborn health science. A lot more needs to be done. Research leads provided by our work need follow on studies.

Vinod Paul
Ashok Deorari

ACKNOWLEDGEMENT

We consider it a great privilege of ours to get the wonderful opportunity to run ICMR Centre for Advanced Research (CAR) in Newborn Health for 5 years. This was an immense opportunity, not only to learn science, research methods, and administrative abilities to run such a venture, but also to work as a team of wonderful individuals who worked in the true spirit of '*Nishkama Karma*', which made working-together full of joy, pleasure and constant learning. We wish to take this opportunity to express our deepest gratitude to one and all who contributed to the success of CAR.

First of all, we wish to thank all of our collaborating partners who intellectually nurtured and painstakingly implemented the research program, namely:

- Professor Siddarth Ramji, Professor S Krishnaprakash, Professor Surinder Kumar, Dr Ashish Jain, Dr Neeraj Gupta, and Dr Manoj Modi from Maulana Azad Medical College;
- Professor KC Agarwal, Dr MS Prasad, Professor Harish Chellani, Professor Manorama Deb, Professor Rajni Gaind, Dr Sugandha Arya, and Dr Sumita Saluja, from VMMC, Safdarjung Hospital;
- Dr Mamta Jajoo, Dr Vikas Manchanda, Dr Vikas Dabbas, and Dr Hitesh Gautam from Chacha Nehru Bal Chikitsalaya;
- Professor Arti Kapil, Professor V Sreenivas, Professor Nandita Gupta, Professor Arun Gupta, Dr Atin Kumar, Dr Manisha Jana, Dr Purva Mathur, Professor Alok Thakkar, Dr Kapil Sikka, Dr Rohit Saxena, Dr T Velpandian, Professor Sheffali Gulati, Dr Biswaroop Chakarbarty, Dr Vandana Jain, Dr Manju Saxena, Dr Anu Thukral, Dr N Chandra Kumar, Ms Madhumati Bose, Ms Savita Sapra, Ms Anuja Agarwal, Ms Savita Saini, and Ms Sumita Gupta from AIIMS
- Dr Sunita Bhatia and Dr Anil Duggal from Kasturba Hospital

Each one of them was available to us whenever we needed them, provided their intellectual inputs, facilitated the implementation, and effectively removed roadblocks during this 5-year journey.

We are grateful to Dr Suvasini Sharma and Dr Naveen Sankhyan for meticulously leading the process of development of 'National Ethics Guidelines for Bio-Medical Research involving Children'. The document has been finally submitted to ICMR for dissemination –thanks to the hard work of both of them.

We are indebted to the ICMR and the team for the most generous financial, technical and administrative support throughout the study period. It was Dr Vasantha Muthuswamy who translated her idea of setting up of CAR in NH at AIIMS with just a few months left of her retirement! Subsequently, the team led by Dr Malabika Roy, Dr Reeta Rasaily, Dr Anju Sinha and Mr VK Bahl worked tirelessly with us to make sure that we completed the project successfully. Despite inherent administrative challenges, they always walked many miles extra to sort out the issues we faced.

We are extremely fortunate to be guided and mentored by a galaxy of eminent scientists, visionaries and, above all, great human beings namely Professor Anand Pandit, Professor ON Bhakoo, Dr Swarna Rekha, and Professor Armida Fernandez. Their ability to think through, farsightedness, deep understanding of scientific issues and a flexible approach guided us what is right, relevant, scientific and doable.

We take this opportunity to express our gratitude to our long-time mentors and guides Professor Vinod Bhutani, Professor Haresh Kirpalani, and Professor Barbara Stoll for their decades of unstinted support to whatever we did, and their valuable inputs at different stages of the projects. We thank Dr Nita Bhandari for her inputs in refining the manuscript on neonatal sepsis.

We are very proud of the high level of quality of research work done as part of CAR. Such a mammoth endeavor cannot be accomplished without a dedicated team working with passion and dedication. We were actually blessed to have one and wish to acknowledge their immense contribution. Indeed, the wonderful team worked tirelessly to achieve the objectives of CAR with highest level of quality.

We wish to thank all the babies and their families, the nurses, the residents, other staff, administrative staff of our respective institutions, our institutional leadership for assisting us at every stage of the CAR. Without their immense support, it would have not been possible for us to carry out this work.

Ramesh Agarwal
M Jeeva Sankar

CAR TEAM

	Chacha Nehru Bal Chikatsalya	Maulana Azad Medical College	Safdarjung Hospital & VMMC	AIIMS (Nodal center)
Principal Investigator (Clinical)	Mamta Jajoo	Siddarth Ramji	K C Aggarwal M S Prasad	Vinod Paul Ashok Deorari
Principal Investigator (Micro)	Vikas Manchanda	S Krishnaprakash	Manorma Deb	Arti Kapil
Co-Principal Investigator	Vikas Dabas Nishant Verma	Surinder Kumar Neeraj Gupta Manoj Modi Ashish Jain	Harish Chellani Sugandha Arya Rajni Gaind	Ramesh Agarwal M Jeeva Sankar
Investigators/ Collaborators			Sumita Saluja	Anu Thukral Arun K Gupta Nandita Gupta Sreenivas V Velpandian T Purva Mathur Rohit Saxena Sheffali Gulati Vandana Jain Atin Kumar Manisha Jana B Chakarbarty Alok Thakar Kapil Sikka Suvasini Sharma Naveen Sankhyan Savita Sapra Sumita Gupta Anuja Agarwala Manju Saksena Ms Savita Saini Ms Madhumati Bose (National Child Trust) *Kasturba Hospital* Dr Sunita Bhatia and Dr Anil Duggal
Senior research officer (SRO)				Shuchita Gupta Suman Chaurasia
RO /SRF	Deepika Manisha Jain	Anil Mor Nidhi Goel Jogendra Singh Nim	Vidya Kumari Charu Manjari Choudhary Kunj Bihari Gupta	Deeksha Mittal Deepali Agarwal Somi Suresh CP Yadav Kaustav Banerjee UK Subhash Das
Psychologist/ Physiotherapist/ Dietitian/				Shivani Purnima Kandpal Surender Singh Garima Dhankar

Technical Assistant				Kamalpreet Kaur Sujata Amrita Saxena Kanak Lata Gupta Geeta Mehto Sandhya Sah Farah Abbasi Raqib Ali Mirdu Sharma Swati B Ankita Kotnala
Assistant				Sant Lal
Accountant				Meenakshi
Research Nurse	Mariya Thomas Jincymol Steffi Wilson Dhanya Alex Priya Sebastian	Simi Thomas Benu Varghese Simi P Johny Benu Varggese Honey May James Rimjhim Johri Dona Jose Shinsa John	Asha Susan Shinsa John Simi P Johny Ann Rainu Jose Vandana Gulati Mariya Thomas Rosemol TM Mary Abraham Jenifer CA Mitali Bhardwaj Anamika	Anjali Thareja Sreemol Sarasan D Vimala Suman Singh Josmi Philip Rekha Rani Angel George Annai Therasa D
Medical Social Worker				Taruna Kaushik Tushara Philip Reena Kuriakose Sukhram Babu Ajay Kumar Singh Faizan Mujeeb
Data Entry Operator	Anchal Wadhwa	Manish Sethi	Nisha Rani Shilhenba Meitei	Akhilesh Sharma Pratibha Gupta
Lab Technician	Zulfikar Ali Ashwini Kumar Abhinav kher	Madan Singh	Mamta Dhooper Deepak Kumar	Neelam Manral Bijoy Jose Vipin V
Lab Attendant/ Attendant		Ashok Kumar	Ashish Kumar Shub Darshan	Shyam Narayan Yadav Dinesh Arya Rajesh Shobhit Sharma Umakant

EXECUTIVE SUMMARY

The ICMR Center for Advanced Research (CAR) for Newborn Health was established at the Department of Pediatrics, All India Institute of Medical Sciences in May 2010. The overall aims of CAR were to undertake clinical research in high priority areas in newborn health and to assist ICMR in capacity development in neonatal health research in the country.

The Center had a mandate to implement eight research projects under five domains over a period of 5 years (2010-15). The projects included the following:

1. *Theme 1: Neonatal sepsis*
   - Project 1: Developing a neonatal sepsis reference centre/registry, and studying molecular epidemiology of bacterial isolates and their antimicrobial resistance pattern
   - Project 2: Preventing newborn infections in neonatal nurseries.[1]

2. *Theme 2: Feeding and nutrition of LBW infants*
   - Project 3: Vitamin D status and need for supplementation among LBW infants

3. *Theme 3: Neurodevelopment follow-up*
   - Project 4: Neurocognitive and physical outcomes of high risk neonates

4. *Theme 4: Capacity building*
   - Project 5: Facilitating development of new multi-site studies on newborn health by researchers of other institutions in the country
   - Project 6: Enhancing research methodology skills of faculty at teaching institutions to stimulate high quality research studies under the postgraduate theses

5. *Theme 5: Technical assistance to ICMR*
   - Project 7: Developing bio-ethics guidelines on child health research in India
   - Project 8: Providing technical assistance to ICMR in neonatal health

Of these, the first two projects were multi-center studies conducted in four major hospitals of Delhi namely, Safdarjung hospital (SJH), Maulana Azad Medical College (MAMC), Chacha Nehru Bal Chikitsalaya (CNBC), and AIIMS. A total of about 70 research staff were employed for different time periods at these four collaborating sites.

A brief summary of the sanctioned projects as well as additional projects is given below:

Developing a neonatal sepsis reference centre/registry, and studying molecular epidemiology of bacterial isolates and their antimicrobial resistance pattern

Objective: To create a comprehensive database for neonatal sepsis that would help to (1) study the epidemiology of sepsis (incidence rates, risk factors, clinical profile, risk factors, etc.) in neonates admitted in four major hospitals of Delhi and (b) undertake phenotypic and molecular characterization of bacterial pathogens causing sepsis

Methods

- We established a network – "Delhi Neonatal Infection Study (DeNIS) collaboration" – comprising the four aforementioned tertiary level hospitals of Delhi.
- A dedicated team of research nurses at each site enrolled all neonates admitted in NICUs and tracked them until discharge/death.
- In neonates suspected to have sepsis, sepsis work-up including cultures of blood and other body fluids was performed as per the Standard Operating Procedures (SOP).
- At each site, two senior neonatologists prospectively assigned the diagnosis of sepsis for each suspected episode after reviewing the clinical course, sepsis screen, and culture reports.
- For molecular typing, we subjected carbapenem resistant isolates of Klebsiella spp. and Acinetobacter spp. to standard phenotyping and genotyping methods for identifying genetic elements imparting carbapenem resistance.
- Strict quality assurance measures covering clinical, microbiology, and data management were implemented at all the sites.

Results

- *Inborn cohort*
  - Of the 88 636 live-births from July 2011 to February 2014, 14 779 neonates required NICU admission; 13 530 (90.0%) neonates were enrolled in the study.
  - Incidence of total and culture-positive sepsis was 14.3% (95% CI 13.8–14.9) and 6.2% (5.8–6.6), respectively. Nearly two-thirds of total episodes occurred at or before 72 h ('early-onset').
  - Two-thirds of isolates were Gram-negative including Acinetobacter spp. (21.9%), Klebsiella spp. (16.6%), and Escherichia coli (13.7%).
  - Majority of the pathogens exhibited high degree of AMR to even 'reserve' antibiotics like extended spectrum cephalosporins and carbapenems; a high proportion of Acinetobacter spp. (81.5%) and Klebsiella spp. (53.8%) was multi-drug resistant (MDR). Colistin resistance was detected in seven (0.7%) Gram-negative isolates.
  - Nearly one-fifth of the deaths were attributable to sepsis.
- *Outborn cohort*
  - Of the 2588 enrolled neonates, sepsis was suspected in 2118 (81.8%), and was confirmed in 1416 neonates (54.7%; 95% CI 52.8 to 56.6); 339 neonates (13.1%) had culture-positive sepsis.
  - Bacterial pathogens accounted for about three-fourths of the isolates. There was a predominance of Gram-negative isolates (52.9%), with common isolates being *Klebsiella pneumoniae* (12.5%), *Acinetobacter baumannii* (11.5%) and *E coli* (8.0%).

- Most *K pneumoniae* and *A baumannii* isolates (70% to 90%) were resistant to carbapenems and were MDR.
- Notably, fungal isolates were responsible for nearly a quarter of systemic infections – mostly in larger neonates (≥ 32 weeks' gestation and/or BW ≥ 1500 g).
- Sepsis was the most common cause of death, accounting for two thirds of total deaths (153/243; 63.0%)

- *Molecular work*
  - Most carbapenem-resistant Klebsiella spp. isolates (81.1%) were found to carry the novel member of metallo β- lactamases i.e. NDM-1.
  - Among carbapenem-resistant *A. baumannii*, a quarter (n=70) of the isolates were NDM-1 positive while seven (3.5%) strains expressed the gene IMP; among the other classes, OXA-23 was expressed in the majority (87.7%), and OXA-58 in a few (10%) isolates.

Interpretation: The high incidence of sepsis and an alarming degree of AMR amongst common isolates underscore the need to understand the pathogenesis of neonatal sepsis and to devise measures to prevent it in low- and middle-income countries

Publications

- The manuscript on inborn cohort is accepted for publication in **Lancet Global Health**;
- The manuscript on outborn cohort is under review in **PLoS One**.
- The two manuscripts on molecular work are about to be submitted to *Scientific Reports* and *Clinical Infectious Diseases* Journal, respectively.

Translational potential

- The process of creating this database has led to convergence of scientists and faculty from various institutes thus establishing an ideal inter-disciplinary platform destined for further collaborative research and innovation in the domain of neonatal sepsis. Three studies have been planned / underway:
- A new study in partnership with THSTI, Gurgaon and NCBS Bangalore "**Understanding disease biology and diagnosis of bacterial sepsis among hospitalized neonates: a multicenter study"** (funded by DBT; 1.84 crores) is already being implemented at AIIMS and SJH; the objectives are to determine the diagnostic performance of procalcitonin and other biomarkers and to undertake exploratory studies including "omics" technology to identify newer biomarkers.
- Another study – "Urinary metabolomic profile of term healthy male neonates from first 24 to 72 hours of life" – is being conducted at AIIMS in collaboration with Department of NMR, AIIMS and ICGEB, New Delhi, to generate normative data for novel biomarkers; the study has been registered as a PhD dissertation and is about to complete recruitment.
- **An ICMR-NIH collaborative study on 'Molecular Epidemiology of Multidrug Resistant (MDR) *Acinetobacter* Infection in Neonates in India'** has been planned in which AIIMS- PGIMER would collaborate with ARLG network institutions in the US.
- Faculty and CAR scientists contributed to development of **global guidelines for reporting studies on neonatal infections (**Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI): an extension of the STROBE statement for neonatal infection research; Lancet Infectious Disease Journal, In press)

Effect of stepwise interventions on hand hygiene compliance rates among healthcare providers: A Before-AND-after study (in lieu of chlorhexidine trial)

Background: The original plan was to conduct a multi-centric RCT on efficacy of whole body application of chlorhexidine in preterm VLBW neonates. Before initiating the trial, we systematically reviewed the literature – evidence from randomized trials of chlorhexidine application showed no significant benefits in risk of mortality or incidence of sepsis; there was paucity of evidence on the safety of chlorhexidine application, particularly in preterm neonates. The updated evidence was presented in the Scientific Advisory Committee (SAC) meeting. The SAC recommended to withdraw the trial and to initiate studies on Quality Improvement (QI) in neonatal sepsis.

Objective: To compare the hand hygiene compliance rates among residents and staff nurses in a level-3 NICU before and after implementation of a stepwise improvement package comprising 4 strategies of interventions namely, standard learning, intensive learning, video monitoring, and video monitoring with feedback

Methods

- The study was conducted at NICU, AIIMS. All bedside nurses and resident doctors involved in routine care of neonates in NICU were enrolled.
- Four research nurses (RN) were trained for recording hand hygiene opportunities and their corresponding actions according to the WHO five moments of hand hygiene; their observations were validated by the PI before the start of the study.
- RN observed the hand hygiene opportunities and actions of providers caring for neonates at randomly selected beds at randomly selected times of each of the three shifts.
- Compliance was defined as the number of positive actions/number of opportunities.

Results

- We observed 7134 opportunities of 55 providers (40 bedside nurses and 15 residents).
- HH compliance improved significantly from 61.8% at baseline to 77% after implementing all the four strategies (relative change 25%, 95% CI: 18% to 32%). The change in HH compliance rates was comparable between the nurses and residents.

Interpretation

- Hand hygiene compliance improved significantly at the end of the stepwise improvement package among nurses and residents in NICU
- An incremental effect on compliance observed with standard learning and CCTV monitoring with individual feedback; CCTV monitoring alone didn't improve the compliance rates much

Publications

- Plan to submit the manuscript in *Pediatrics* Journal
- Systematic review on whole body chlorhexidine cleansing published in *The Pediatric Infectious Disease Journal*
- Chandrasekaran A et al. Topical umbilical cord care. *Pediatr Infect Dis J* 2013;32:801 (Letter to editor)

Next steps

- Submit the following proposals for funding to National/International agencies:
  - Quality Improvement for reducing the incidence of HAIs in major hospitals of Delhi
  - Developing a QI Collaborative Network for sepsis in SNCUs with bench marking and support by level-3 neonatal units (including AIIMS, MAMC, PGI, etc.)

Vitamin D status and need for supplementation among LBW infants

Background: As per the original proposal, we were to undertake studies in two phases to determine (i) the prevalence of vitamin D deficiency (VDD), and (ii) the optimal supplemental dose of vitamin D for prevention of VDD in LBW infants. In the meantime, we had completed another study – done in 3 hospitals in Delhi and funded by ICMR – that found a high prevalence of VDD in LBW infants (n=220) as well as normal birth weight (NBW, n=119) infants at birth (87.3% and 88.6%, respectively), and at around 3 months of age (60.6% and 71.6%, respectively). Given the similar objectives of the proposed phase-1 study under CAR, the SAC advised us to drop it and proceed directly to the study on evaluating the optimal supplemental dose of vitamin D. Accordingly, we undertook one RCT:

Hypothesis: Among preterm infants (28 to 34 weeks' gestation), daily supplementation with 800 IU of vitamin D3 would reduce prevalence of VDD (serum 25(OH)-vitamin D_3 <20 ng/mL) by 50% at 40 weeks' PMA when compared to daily supplementation with 400 IU

Objectives: To compare the prevalence of VDD following daily supplementation with 800 IU vs. 400 IU of oral vitamin D3 at 40 weeks' PMA

Methods: All enrolled infants were randomized to receive either 800 IU/day or 400 IU/day of vitamin D from 2 weeks of age until 3 months' corrected age (CA); they were followed-up monthly to ensure compliance with treatment and for clinical signs of rickets. Serum calcium, phosphate, alkaline phosphatase, vitamin 25(OH)D, and parathyroid hormone (PTH) were estimated at 3 months' CA; bone mineral density (BMD) was assessed using DEXA.

Results: We enrolled 96 infants in the study (n=48 in both groups). Prevalence of VDD in 800-IU group was significantly lower than that in the 400-IU group at 40 weeks (38.1% vs 66.7%; RR: 0.57; 95% CI: 0.37–0.88) and at 3 months' CA (12.5% vs 35%; RR: 0.36; 95% CI: 0.14–0.90). One infant (2.4%) in the 800-IU group had vitamin D excess (100–150 ng/mL). Bone mineral content and BMD were not different between the groups.

Publications/achievements

- Manuscript got published Pediatrics Journal (Natarajan CK et al. 2014;133:e628–34; Impact Factor: 5.5)

Study won NNF Gold Medal for the 'Best research paper' at NNF Annual Meeting 2012; American Academy of Pediatrics (AAP) had chosen it for public release during PAS meeting in May 2013.

Additional studies on vitamin D

Given the importance of the topic and the paucity of studies, we undertook two additional studies to determine the optimal dose of vitamin D supplementation in *term healthy* infants.

Prevalence of vitamin D deficiency following supplementation with 400 IU/day in term infants

Background & objectives: In view of severe nature of VDD, the recommendation of 400 IU/day by international bodies like AAP and IAP is unlikely to be sufficient for Indian neonates. We sought to evaluate the prevalence of VDD at 3 months in term healthy infants supplemented with daily Vitamin D (400 IU) from birth.

Methods: Healthy term infants (n=111) were enrolled at birth and started on vitamin D (oral 400 IU) daily. Primary outcome was prevalence of VDD (25(OH) <20 ng/mL) and vitamin D insufficiency (VDI; 25(OH) 20-29 ng/mL) at 14 ± 2 weeks of age.

Results: VDD (25(OH) <20 ng/mL) was present in 47 (52.2%) infants at 3 months of postnatal age. Clinical and radiological features of rickets were detected in four and one infants, respectively. There was no instance of vitamin D excess or toxicity.

Interpretation: The prevalence of VDD and VDI continues to be high at 14 weeks of age in term healthy infants in India despite daily oral dose of 400 IU of vitamin D3, possibly indicating it as suboptimal dose.

Publications: The manuscript is being submitted for publication in *Pediatrics* Journal.

Prevalence of vitamin D deficiency following supplementation with 800 IU/day in term infants

Background & objectives: As we found in the previous study, daily supplementation of 400 IU vitamin D for healthy term infants was not sufficient for achieving vitamin D sufficiency in Indian infants. We planned to evaluate if supplementation of 800 IU/day from birth would reduce the prevalence of VDI at 6 months of age.

Methods: In this prospective interventional study, we supplemented 800 IU/day of vitamin D in term healthy infants (n=70) starting within 48 hours of birth and continued until 6 months of age. Serum 25(OH)D was measured at birth and 6 months for all infants.

Results: VDD was present in 4 (6.9%; 95% CI 1.9 - 16.7) of 58 infants followed-up until six months of age. However, four infants (6.9%) developed vitamin D excess (25(OH)D 100-150 ng/mL) at any point during 6-months follow-up and required reduction of the daily dose of supplementation. No infant developed vitamin D toxicity (25(OH)D>150 ng/mL).

Additional studies on feeding and nutrition of LBW infants

Complementary feeding at four versus six months of age in preterm infants: a randomized, multicentre trial *(PhD thesis of Dr Shuchita Gupta, Scientist-3, supported by ICMR-CAR)*

Background: There is paucity of evidence on optimal time of initiation of complementary feeding (CF) in preterm infants.

Objectives: To examine the effect of initiation of CF at four vs. six months of corrected age (CA) on weight-for-age z-score at 12 months' CA (WAZ_{12}) among preterm infants (<34 weeks' gestation).

Methods: An open-label randomized trial was conducted at AIIMS, Safdarjung Hospital, and Kasturba Hospital. Infants <34 weeks' gestation (n=403) were randomized at 4 months' CA (1:1) to receive CF at 4 months' CA (4-month group), or at 6 months' CA (6-month group), using computer generated randomization schedule of variable block size.

Results:

- We did not find any difference in WAZ_{12} at 12 months' CA between the two groups (-1·6±1·2 vs. -1·6±1·3; mean difference 0·005, 95% CI -0·24 to 0·25).
- There were more episodes of hospitalization in 4-month compared to the 6-month group (episodes per infant-month 0·23 vs. 0·13; incidence rate ratio 1·8, 95% CI 1·0 to 3·3).
- Other secondary outcomes like body composition, bone mineralization status, iron stores, and markers of metabolic syndrome at 12 months' CA were comparable.

Interpretation: The study suggests that initiation of CF at six months' CA is preferable to four months' CA in infants less than 34 weeks of gestation.

Neurocognitive and physical outcomes of high risk neonates

Objective: To determine the neurocognitive and physical outcome of preterm and term low birth weight (LBW) infants and neonates with perinatal asphyxia at 18 months of age

Methods: Eligible infants were enrolled during birth hospitalization in five categories:

1. Very preterm (≤32 weeks or BW≤1500 g)
2. Late preterm (gestation 34-36 weeks)
3. Term SGA (BW for gestational age <10th centile)
4. Term asphyxiated (≥37 weeks and requiring positive pressure ventilation >1 minute at birth), and
5. 'Control' comparator group of term healthy infants (BW between 10^{th} and 90^{th} centile and no NICU stay)

Primary outcome was major disability defined as presence of cerebral palsy or developmental delay or hearing or vision impairment or epilepsy, at 18 months of age and secondary was growth.

Results:

- A total of 820 infants were enrolled: 212 very preterm, 254 late preterm, 87 term SGA, 17 term asphyxiated, and 250 term healthy infants. Of these, a total of 776 infants (follow-up rate of 94.6%) were assessed at 18 months of age for the final outcome.
- The primary outcome – major disability – was observed in 11.3% of very preterm and 5.4% of late preterm infants. Among term infants, the rates of disability were lower: 6.0% of term asphyxiated, 3.7% of term SGA and 2.9% of term AGA infants had disability at 18 months of age.
- There was little catch-up growth (defined as change in growth parameter SDS scores of >0.67 over time) in weight, length and head circumference among very and late preterm infant cohorts (catch-up in weight: 11.8% and 23.0%; length 19.9% and 23.0% in very preterm and late preterm, respectively).
- A higher proportion of infants in term SGA cohort had undernutrition (weight for age z-score < -2 SDS) and stunting (length for age z-score < -2 SDS) at 18 months of age compared to very preterm and late preterm infants.
- On multivariate regression analysis with major disability as dependent variable and clinical features significant on univariate analysis as predictors, BW<1500g, monthly family income <INR 7323 and having required mechanical ventilation during birth hospitalization were found to be significant predictors of major disability.

Interpretation:

- In a tertiary care setting with adequate quality of care during NICU stay as well as post-discharge follow-up clinic, it is feasible to achieve low rates of disability even among high risk preterm infants
- Postnatal growth of preterm and term SGA infants continues to remain an important concern, with few of the preterm infants showing catch-up growth.

Additional studies on follow-up and development of high-risk infants

Challenges of implementing universal newborn hearing screening (UNHS) at a tertiary care centre

Background: There is limited data on the cost-effectiveness of UNHS, especially at places where there is no system linking the screening and diagnostic assessments with appropriate rehabilitation services.

Objectives: To report experience of implementing UNHS, identify risk factors associated with failed two-step automated acoustic brainstem response (AABR) and evaluate the cost of AABR.

Results:

- Screening coverage was moderate (84 %), with 2265 of total 2700 eligible infants screened with initial AABR. A total of 273 of 2265 infants were "refer" on first screen. Second screen was done on 233, of which 58 were "refer". Of these, 35 underwent conventional ABR, of which 5 were diagnosed to have hearing impairment. Only 2 could get hearing aid.
- Overall, a total of 2197 (81.4%) infants passed, 496 (18.4%; excluding 2 deaths) were lost to follow up at various stages, and 5 (0.2 %) were diagnosed with hearing impairment, all of whom were high risk.
- Average cost of AABR was INR 276 per test.

Interpretation: UNHS is feasible to implement, but significant lost to follow up and non-linkage with appropriate rehabilitation services limit its utility.

Diagnostic utility of oto-acoustic emissions for hearing screening of high risk neonates

Background: There is paucity of robust data on the diagnostic utility of otoacoustic emissions (OAEs) among high-risk neonates.

Objectives: To determine the diagnostic performance of distortion product OAE (DPOAE) and transient evoked OAE (TEOAE), with two-step AABR as gold standard to determine hearing loss at 40 dB

Results: High risk infants (n=72) identified at AIIMS-NICU underwent simultaneous hearing screening using TEAOE, DPOAE and AABR prior to discharge. The sensitivity of DPOAE and TEOAE were 13% and 52.2%, respectively, and the specificity were 98.3% and 91.6%, respectively.

Interpretation: OAEs have low sensitivity and high specificity for hearing screening among high risk neonates, when compared to a two-step AABR, with TEOAE performing better than DPOAE. The results primarily serve as pilot data to inform further studies to test the diagnostic utility of OAEs/protocols using OAEs among high-risk neonates.

Reducing post-discharge mortality in preterm neonates

Background: We noticed a high post-discharge mortality (70/371; 18.9%) at one of the participating sites in CF trial. Majority of deaths were among VLBW infants (n=56; n=80.0%) and within one month of discharge prior to their first follow-up visit with the research team (RT).

Methods: We did a quality improvement activity involving: (1) an earlier follow up at two instead of six weeks of age for infants with birth weight of 1500 g or lesser, (2) provided families with contact numbers of RT for telephonic consultation on 24x7 basis.

Results: During post-intervention period, we noted that of 241 (87.3%) of 276 discharged neonates, 29 (12.0%) died – an absolute reduction of 6.9% (95% CI 1.1–12.6%, p=0.02) compared to pre-intervention period (adjusted OR 0.54, 95% CI 0.29–0.98).

Interpretation: The high post-discharge mortality among preterm VLBW infants may be reduced by timely empowerment of the family coupled with improving access to healthcare through 24x7 telephonic support, which would be of significant importance in resource constrained settings with limited home follow up.

Facilitating development of new multi-site studies on newborn health by researchers of other institutions

Background: The ICMR supported the establishment of the National Neonatal-Perinatal Database Network of 18 leading neonatal care institutions since 1989 providing information of largest cohort of 150,000 neonates in India. We proposed to take this process forward as a catalyst.

Methods: We organized two protocol development meetings of the researchers of the Network along with ICMR scientists and epidemiologists.

Outputs: We facilitated development of the following multisite studies:

1. Efficacy and safety of indigenous goat lung surfactant extract for treatment of RDS in preterm babies (Dr Ramesh Agarwal, AIIMS, New Delhi). The study was funded by Wellcome Trust, UK (10.4 crores; 2014-17) and being undertaken at 13 sites in India.
2. Developing postnatal growth standards for Indian very low birth weight infants (Dr Swarna Rekha Bhat, St John's Hospital, Bangalore)
3. Establishing a national neonatal network for quality improvement (Dr Deepak Chawla, GMCH, Chandigarh)

Enhancing research methodology skills of faculty at teaching institutions to stimulate high quality research studies under the postgraduate theses

Objectives: To improve the quality neonatal-perinatal research through capacity strengthening in the teaching institutions of the country

Methods and outputs: Using standardized resources, we conducted two interactive, hands-on research methodology workshops – one each in Delhi and Bangalore – to help young faculty (n=75) working in medical and nursing colleges in India. In a survey undertaken one year later, the participants (n=45) expressed immense satisfaction on the objectives and processes of the workshop. The proportion of participants able to secure extramural funding increased from 7 (15.6%) to 13 (28.9%) after attending the workshop.

The SAC advised us to develop an e-repository for research methodology that can be used for training faculty and other health providers across the country. We have compiled the relevant study materials including PowerPoint presentations and key articles. The same has been made available to ICMR to upload on ICMR website.

Developing bio-ethics guidelines on child health research in India

Objectives: To create bioethics guidelines for conduct of research involving newborn and child participation including drug trials.

Methods and output:

- The existing ICMR ethics guideline for research on human subjects (2006) has only a small section pertaining to research in children. The need was felt to develop more comprehensive detailed guidelines which pertain to the specifics of ethics in biomedical research in neonates and children.
- We developed bio-ethics guidelines on child health research in India through a process involving desk review of existing national and international guidelines, expert group consultation, peer review process and by inviting suggestions and comments from different stakeholders through a wider consultation.

The bioethics document covers the ethical and legal issues that researchers need to consider when carrying out biomedical research in neonates and children. These easy-to-read and pragmatic guidelines provide general principles that can be applied in most situations. These guidelines are meant for use by researchers, ethical committees and other involved stakeholders.

Providing technical assistance to ICMR in neonatal health

The Division has been providing technical assistance to the ICMR in different domains related to newborn and child health in India. The PI and co-PIs serve as key members of various committees of ICMR – PRG on Prematurity, India Neonatal Infections study (INIS) group, Task force project on Home-based Management of Young Infants, community based KMC study, etc.

Additional outputs generated by faculty and CAR staff using CAR resources

1. ICMR CAR staff conducted several systematic reviews for WHO that culminated global guidelines on postnatal care of mothers and newborns, simplified antibiotics regimens for pSBI in young infants, and care of preterm infants and 6 publications in Journal of Perinatology (list provided in 'Output' section)
2. Dr T. Velpandian developed standardized chlorhexidine wipes and are being clinically validated in adult population**.**

OUTPUTS

S. No	Items
	Journal articles - published
1.	Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration. Unusual profile and high antimicrobial resistance among pathogens of sepsis in inborn neonates in tertiary care centres: Findings from a multi-site cohort study in Delhi. Accepted for publication in ***Lancet Global Health***
2.	Natarajan CK, Sankar MJ, et al. Trial of daily vitamin D supplementation in preterm infants. **Pediatrics** 2014;133:e628-34
3.	Sankar MJ, Paul VK. Efficacy and Safety of Whole Body Skin Cleansing with Chlorhexidine in Neonates – A Systematic Review. ***Pediatr Infect Dis J*** 2013;32(6):e227-34.
4.	Agarwal R, Virmani D, et al for Investigators of LBW Micronutrient Study Group. Vitamin D Status of Low Birth Weight Infants in Delhi: A comparative study. ***J Trop Pediatr*** 2012;58:446-50.
5.	Agarwal R, Virmani D, et al for Investigators of LBW Micronutrient Study Group. Poor Zinc Status in Early Infancy among Both Low and Normal Birth Weight Infants and Their Mothers. ***Neonatology*** 2013;103:54-9
6.	Agarwal R, Virmani D, et al for Investigators of LBW Micronutrient Study Group. Iron Stores in Low and Normal Birth Weight Infants at Birth and in Early Infancy. ***Indian J Pediatr*** 2013 Aug 27. [Epub ahead of print]
7.	Gupta S, Sah S, et al. Challenges of Implementing Universal Newborn Hearing Screening at a Tertiary Care Centre from India. ***Indian J Pediatr*** 2015 Feb 6. [Epub ahead of print]
8.	Saha B, Jeeva Sankar M, et al. Iron Stores in Term and Late Preterm Small for Gestational Age and Appropriate for Gestational Age Neonates at Birth and in Early Infancy. ***Indian J Pediatr***. 2015 Dec 15 [Epub ahead of print]
9.	Gupta S, Chaurasia S, et al. Neonatal research in India: current status, challenges, and the way forward. ***Indian J Pediatr***. 2014 Nov;81(11):1212-20
10.	Fitchett EJA, Seale AC, et al. Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI): an extension of the STROBE statement for neonatal infection research. Accepted for publication in ***Lancet Infect Dis***
11.	Seale AC, Head MG, et al for SPRING. Neonatal infection: a major burden with minimal funding. ***Lancet Glob Health*** 2015;3(11):e669-70
12.	Sankar MJ, Chandrasekaran A, et al. Umbilical cord cleansing with chlorhexidine in neonates: a systematic review. ***J Perinatol*** 2016;36 Suppl 1:S12-20
13.	Sankar MJ, Chandrasekaran A, et al. Vitamin K prophylaxis for prevention of vitamin K deficiency bleeding: a systematic review. ***J Perinatol*** 2016;36 Suppl 1:S29-35
14.	Sankar MJ, Gupta N, et al. Efficacy and safety of surfactant replacement therapy for preterm neonates with respiratory distress syndrome in low- and middle-income countries: a systematic review. ***J Perinatol*** 2016;36 Suppl 1:S36-48
15.	Natarajan CK, Sankar MJ, et al. Surfactant therapy and antibiotics in neonates with meconium aspiration syndrome: a systematic review and meta-analysis. ***J Perinatol*** 2016;36 Suppl 1:S49-54
16.	Thukral A, Sankar MJ, et al. Efficacy and safety of CPAP in low- and middle-income countries. ***J Perinatol*** 2016;36 Suppl 1:S21-8
17.	Das RR, Sankar MJ, et al. Is the Practice of "Bed share" Beneficial and Safe to Neonates: A Systematic Review. ***Int J Pediatr*** 2014;2014:468538
18.	Sankar MJ, Natarajan CK, et al. When do newborns die? A systematic review of timing of overall and cause-specific neonatal deaths in developing countries. ***J Perinatol*** 2016;36 Suppl 1:S1-S11
19.	Chandrasekaran A, Sankar MJ, et al. Topical umbilical cord care. ***Pediatr Infect Dis J*** 2013;32:801
	Journal articles – submitted/to be submitted
20.	Gupta S, Agarwal R, et al. Complementary feeding at four versus six months of age for improved growth of preterm infants: a multi-centre RCT. Submitted for publication in *N Engl J Med*
21.	Jajoo M, Manchanda V, et al for Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration. Alarming rates of antimicrobial resistance and fungal sepsis in outborn neonates. Submitted for publication in *PLoS One*
22.	Shridhar S, Sankar MJ, et al. Impact of stepwise interventions for improving hand hygiene compliance

	among health-care: a before-and-after study. To be submitted for publication in *Pediatrics*
23.	Gupta S, Agarwal R, et al. Neurocognitive and physical outcomes of very preterm, late preterm, and term small-for-gestational age infants. To be submitted for publication in *Arch Dis Child*
24.	Chaurasia S, Sankar MJ, et al. Formulating rational antibiotic policy in an NICU- a stepwise approach. To be submitted for publication in *Ind Pediatr*
25.	Molecular epidemiology of carbapenem resistant *Klebsiella pneumoniae*: a multi-centre study. To be submitted for publication in *Scientific Reports*
26.	Molecular epidemiology of carbapenem resistant *Acinetobacter baumannii*: a multi-centre study. To be submitted for publication in *Clin Inf Dis*
27.	Comparative evaluation of cranial ultrasound and MRI at term equivalent age for pred n iction of neurodevelopmental outcome. To be submitted for publication in *Pediatrics*
	National guidelines
28.	National Ethics Guidelines for Bio-Medical Research involving Children
	Contributions to WHO guidelines
29.	WHO recommendations on intervention to improve pre-term birth outcomes
30.	WHO recommendations on postnatal care of the mother and newborns 2013
31.	Managing possible serious bacterial infections in young infants when referral is not feasible
	Workshops
32.	Two research methodology workshops – one each in Delhi & Bengaluru
33.	One research methodology workshop (1-day) in Neocon 2014 at Patna, Bihar
	Career development – PhD/others
34.	Trained two PhD scholars under CAR: one (Dr S Gupta) has completed her PhD; another one (Dr S Chaurasia) is currently on roll
	Contributions to books
35.	Chapter: Follow up of High-risk infants- PG Textbook of Pediatrics
36.	Chapter: Neonatal Sepsis- PG Textbook of Pediatrics
	Learning resource material
37.	e-repository for Research methodology: power-point presentations and reading material
38.	Toolkit: follow up of high risk infants including 'early intervention' strategies
39.	How to draw blood culture: video
40.	How to perform lumbar puncture: video
41.	How to initiate complementary feeding: video
	Technical support
42.	ICMR: On newborn sepsis study; AMR; LBW feeding GoI: Policy on Chlorhexidine, KMC, Antenatal steroids, ANM using gentamicin, vitamin K

RESEARCH LEADS

Themes	Inferences drawn	Emerging hypotheses/questions	Leads followed
#1: Neonatal Sepsis	• High mortality rate despite early treatment • EOS twice as common as LOS • No difference between the risk factors of EOS and LOS • High incidence of AMR • High prevalence of fungal infection in referred neonates • Hand hygiene compliance rates can be augmented using various modalities, even in busy centres • Success of strategies can be ensured when they are coupled with a motivational focus and increasing knowledge base	• Which factor(s)- the host (susceptibility), agent (virulence) or disease biology (complex interaction) - drive the apparent sepsis picture revealed by the study • What existing or novel markers/signatures of sepsis can reliably diagnose it early, especially at point-of-care to timely reduce this huge burden • What could be the potential low-cost interventions to prevent and adjunctive treatment to improve sepsis outcomes • Whether our QI model replicated in level I/II settings including SCNUs can reduce prevalence of sepsis • What is the burden and degree of long-term sequelae/disability due to sepsis in the survivors in our settings and how targeted early intervention measures can be implemented effectively	• Exploratory studies in the biology component of the DBT funded study is one of the steps to address these questions • NIH collaboration aims to decipher the AMR determinants linked to the most common pathogen in the study- *A. baumannii* • Several gripping questions on pathogenesis and captivating ideas on AMR deserve larger or longer collaborative research
#2: Feeding and nutrition	• It has been established that Indian infants (both preterm and term) have high degree of Vitamin D Deficiency (VDD) • Optimal doses of supplementation is not clear yet, since high doses put significant infants at risk of toxicity that is unevaluated yet • Early introduction of complementary feeding (CF) has no specific advantage (rather puts at risk), and iron stores and bone mineralization remain poor in general	• What optimal dosing for Vitamin D supplementation would safely achieve sufficiency • Which interventions would most efficiently improve VDD at low-cost (including maternal food fortification) and what strategy would make these potentially scalable at community level • Since early CF along with optimal iron supplementation was not enough, what strategies be devised or modalities be identified to enhance nourishment as well as improve growth outcomes	Concept note under planning
#3: Neurodevelop-mental follow up	• Though disability rates were lower in general, optimal growth remains a matter of concern • This is more so in a preterm and term SGA infants who remain undernourished and stunted at 18 months' age • Reducing disability rates in sub-cohort of extremely premature neonates or ELBWs remains more challenging • Simple interventions like regular telephonic support can substantially	• How 'early intervention' programs can be institutionalized at different levels of care • What optimal follow-up frequency is needed to pick up early signs of sequelae in order to ensure intact growth • Whether the program for such follow up should have basic and advanced components (for suitable implementation depending on the level of care) and how technical/expert resources could be channelized to support such programs • What are the barriers to implementation of such simple	A protocol with embedded 'early intervention' strategies for long-term follow up (six years) study is under development

	bring down the post-discharge death/disability rates among NICU graduates	interventions and how to scale these up in a cost-effective manner	
#4: Capacity building	• Training and mentoring of the young medical faculty in the workshops has helped in better research outputs from the respective units/hospitals	• Whether local institution-based model can be identified for effectively promoting good quality research and encouraging (translational) outputs • Whether online or ready-access to expert mentorship and resource material supplying channel help facilitate the above model better	In-house discussion meeting planned

PROJECT OVERVIEW

1. Project title: ICMR Advanced Centre for Newborn Health Research

2. PI (name & address)

Dr Vinod K. Paul
Professor & Head, Department of Pediatrics
All India Institute of Medical Sciences, New Delhi

Dr Ashok K. Deorari
Professor, Department of Pediatrics
All India Institute of Medical Sciences, New Delhi

3. Collaborating sites

Maulana Azad Medical College and Lok Nayak Hospital, New Delhi
Safdarjung Hospital and Vardhman Medical College, New Delhi
Chacha Nehru Bal Chikitsalya, Geeta Colony, New Delhi

4. File No. / OPA No.

5/7/305/08-RHN

5. Date of start

15th May, 2010

6. Duration

5 years (15th May 2010 to 14th May 2015); later, duration extended until 14 Nov 2015

7. Total budget

INR 8,94,31,177/-

8. Objectives of the proposal

Followings are the broad objectives of the Centre:

1. To generate quality evidence of clinical/program relevance on key neonatal health issues
   i. Neonatal sepsis
   ii. Feeding and nutrition of LBW infants
   iii. Neurodevelopment outcome of high risk neonates
2. To contribute toward capacity development in neonatal health research
3. To provide technical assistance to the ICMR in selected areas

THEMES & PROJECTS –

METHODS & KEY RESULTS

THEME 1: NEONATAL SEPSIS

Project 1: Developing a neonatal sepsis reference centre/registry, and studying molecular epidemiology of bacterial isolates and their antimicrobial resistance pattern

Deviations

None

Objectives

To create a comprehensive registry for neonatal sepsis that would help to

- Evaluate the epidemiology of sepsis including burden and incidence rates, clinical and microbiological profile, risk factors, etc.) in neonates admitted in four major hospitals of Delhi
- Undertake phenotypic and molecular characterization of bacterial pathogens causing neonatal sepsis

Methods

- This prospective cohort study was conducted in four hospitals of Delhi, namely Chacha Nehru Bal Chikatsalya (CNBC), Maulana Azad Medical College (MAMC), Safdarjung Hospital, and All India Institute of Medical Sciences (AIIMS, Nodal centre).
- The study was conducted in three phases – passive surveillance for collecting information on bacterial isolates (Phase I), active surveillance to estimate the incidence of neonatal sepsis (Phase II), and characterization of molecular epidemiology of the bacterial isolates including the determinants of their antimicrobial resistance (Phase III).
- In phase I, we developed structured forms for data collection, formulated consensus guidelines for screening/diagnosis, and standardized the procedures for cultures and other investigations.
- ***Enrolment:*** All neonates admitted to NICU and suspected to have sepsis were enrolled in the study. Sepsis was suspected in the presence of perinatal risk factors or a set of perinatal risk factors and/or clinical signs adapted from CDC NHSN criteria and Young Infant Study Algorithm (Appendix, Table 1).
- ***Clinical work-up:*** Cultures of blood and other sterile body fluids were taken for before starting antibiotics in all enrolled neonates (Figure 1). The research staff performed or assisted in investigations as per the pre-agreed protocol. Blood was collected in Trypticase Soy broth (TSB; Diffco, India) while CSF was collected in sterile screw cap vials. The culture samples were transported to the research laboratory immediately after collection or kept in the onsite incubator maintained at 37^{o}C till transport.
- ***Laboratory work-up:*** The causative organisms were identified by standardized conventional culture methods in the microbiology laboratory (Appendix, Panel 1). In the outborn unit (CNBC), the samples were processed using an automated system – Bactec 9120/ Bactex FX 200 (Biomeriux, France) and antimicrobial susceptibility testing (AST) was performed using Vitek 2 compact system (B^{i}omérieux, France). Dedicated research team processed the samples under the guidance of site PI/co-PI.
- ***AST:*** A uniform panel of antibiotics (Oxoid, UK) was used across all the sites. Results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (2011-13). The organisms were labelled as susceptible (S), intermediate (I), resistant (R), or not tested for each individual antibiotic. Resistance (I or R) was categorized further according to antibiotic classes: (i) extended spectrum cephalosporins (resistance to any two of the three third generation cephalosporins namely, ceftazidime, cefotaxime, or ceftriaxone) (ii) aminoglycosides (resistance to any of the three: amikacin, gentamycin, or netilmycin) (iii) carbapenems (resistance to either meropenem or imipenem) (iv) fluoroquinolone (resistance to ciprofloxacin) and (v) piperacillin-tazobactum. If resistance to any three of the five antibiotic classes was detected, the pathogens were labelled as multidrug resistant (MDR).

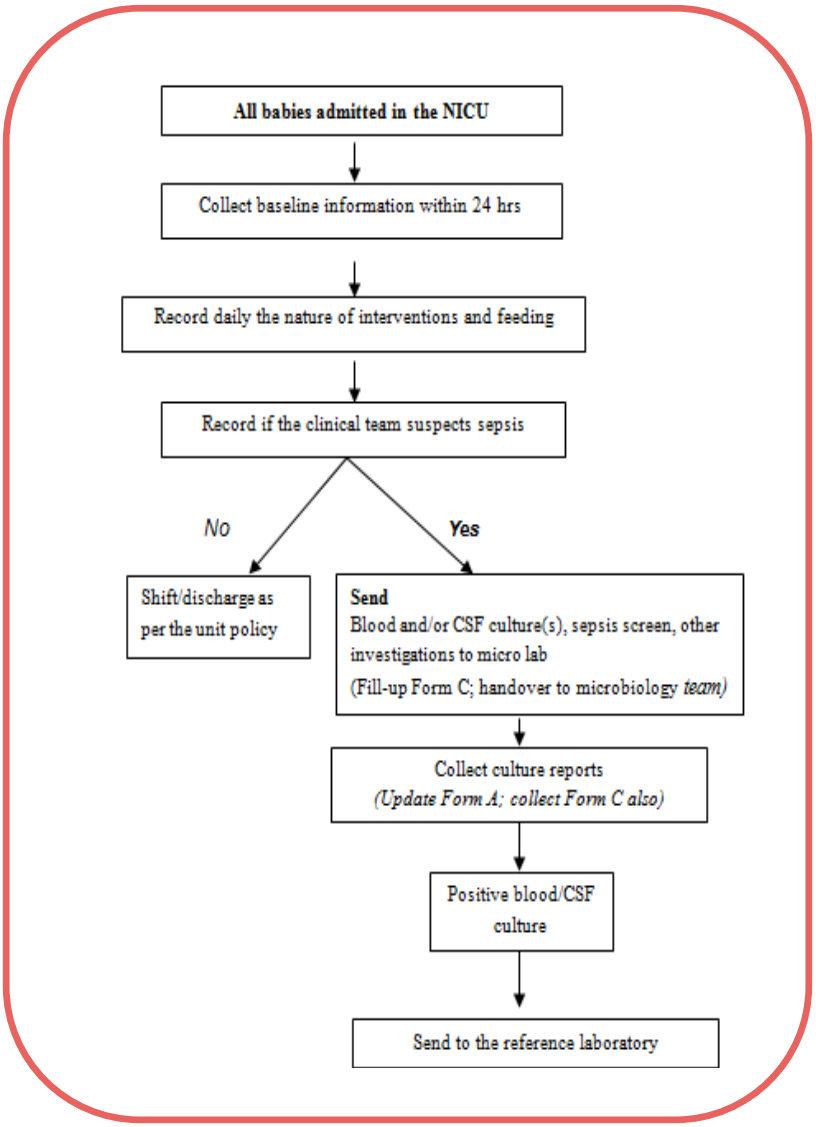

Figure 1: Study plan

- ***Molecular typing of resistance genes***: Carbapenem resistance was the focus of the molecular work which involved the two common organisms, *Klebsiella pneumoniae* and *Acinetobacter baumannii* pooled from all the 4 sites.
    - *Phenotyping:* The AST results were first confirmed by commercially available Etest (biomeriux, France) for meropenem and imipenem breakpoints (CLSI 2011-12); the resistant phenotypes were further tested for carbapenemase production by Modified Hodge test (MHT) and Metallo B Lactamases E-test (biomeriux, France).
    - *PCR screening for carbapenemases:* After extracting the bacterial DNA using Qiagen DNA mini kit, all carbapenem resistant isolates confirmed by MHT and /or MBL E-test were screened for the presence of *blaNDM*-1, *blaVIM, blaIMP* and *blaKPC* genes in multiplex PCR (Appendix Panel 1). Additionally, *Acinetobacter* spp. isolates were tested for oxacillinase genes.
    - *DNA sequencing:* The amplified product was purified (Appendix Panel 1), which was run on 3130XL genetic analyzer (Life technologies, USA). The deducted DNA sequence was aligned using Genedoc software (Nicholas and Nicholas, version 2.12) and the final read was submitted in the gene database for generating accession number.
- ***Quality assurance - clinical:*** A senior neonatologist at each site (PI/co-PI) prospectively assigned the diagnosis of sepsis based on clinical course, sepsis screen results, and culture reports. Extensive quality assurance measures were set-up including detailed SOPs, dry run of four weeks for finalizing the case record forms (CRF) and SOPs, and cross-checking of CRFs daily for accuracy and completion. Data was entered in duplicate at real-time in an online database – developed in Visual Basics as front-end and MS SQL server as back-end with inbuilt range and logical checks (Appendix Table 2).

- ***Quality assurance - microbiology:*** Research microbiology units used specially procured high-quality culture media and antibiotics disks. As a part of external quality assurance scheme (EQAS), identification and AMR pattern of 10% isolates were cross-checked at a different participating site.
- ***Data analysis***
  - Statistical analysis was performed using Stata 11.2 (Appendix Panel 1).
  - For analysis of risk factors of sepsis, we included neonates with culture positive sepsis in the first 72 hours (for EOS) or between 73 hours and 28 days of life (for LOS) as 'cases', and those in whom sepsis was neither suspected nor diagnosed at any time in the first 28 days of life as 'controls' (Appendix Table 3).
  - Only maternal and perinatal risk factors were considered for EOS; for LOS, only neonatal care practices such as duration of IV cannula, parenteral nutrition, or mechanical ventilation, and so on were included as 'antecedent' factors, achieved by truncating the information until the day before the onset of first episode of sepsis.
  - We used multivariate model–stepwise logistic regression with forward selection–to determine the final set of risk factors (after first estimating the gestational age and birth weight and study site adjusted risk for predictors by logistic regression; Appendix Panel 1). The discriminatory power of the final model was evaluated using the area under the receiver operating characteristic curve.

Key results

A) Inborn cohort

- A total of 88 636 live births occurred from 18th July 2011 to 28th February 2014. Of them, 14 779 (16·7%) neonates required NICU admission. After excluding neonates who were recruited in a concurrent trial (n=1249), a total of 13530 (90·0%) were enrolled in the study (n= 9239, 2657, and 1634 at the three sites) (Appendix Figure 2 Study flow).
- The mean birth weight and gestation were 2211±741 g and 36·0±3·4 weeks, respectively. Approximately two-thirds of neonates (n=8111, 59·9%) were low birth weight; nearly half (n=5989, 44·2%) were preterm (Appendix Table 4)
- ***Incidence of sepsis***
  - A total of 4650 episodes of sepsis were suspected and 7131 cultures were performed in 4408 neonates. Final diagnosis of sepsis was assigned in 1980 episodes (1934 neonates).
  - The incidence of total sepsis and culture-positive sepsis was 14.3% (95% CI 13.8–14.9) and 6.2% (5.8–6.6; Table 3) of NICU admissions. The incidence density of total and culture-positive sepsis was 24.6 and 10.5 per 1000 patient-days, respectively (Table 3; Appendix Table 5 for site-wise details).

Table 3: Incidence and case fatality of neonatal sepsis

N	Total sepsis	Culture positive sepsis	Culture negative sepsis	Meningitis
Incidence*: number of neonates (%; 95% CI)				
13 530	1934 (14·3%; 13·8–14·9)	840 (6·2%; 5·8–6·6)	1094 (8·1%; 7·6–8·6)	200 (1·5%; 1·3–1·7)
Incidence density: number of episodes (density per 1000 patient-days; 95% CI)				
80 427	1980 (24·6; 23·6–25·7)	847 (10·5; 9·8–11·3)	1133 (14·1; 13·3–14·9)	200 (2·5; 2·2–2·8)
Case fatality rate: number of neonates (%; 95% CI)				
–	496/1934 (25·6%; 23·7–27·7)	400/840 (47·6%; 44·2–51·0)	96/1094 (8·8%; 7·2–10·6)	102/200 (51·0%; 43·8–58·1)

*Data expressed as n (%); * among those admitted in the NICUs*

- ***Profile of pathogens***
  - Of the total 1005 isolates, about two-thirds were Gram-negative pathogens, the most common being *Acinetobacter* spp. (22·1%), *Klebsiella* spp. (16·8%), *Escherichia coli* (13·6%), and *Pseudomonas* spp. (6·8%) (Figure 4; site-wise profile: Appendix Table 6).

- The predominant Gram-positive pathogens were coagulase negative staphylococci (CoNS; 14·9%), *Staphylococcus aureus* (12·1%), and *Enterococcus* spp. (5·6%).
- The pathogen mix in early-onset sepsis (EOS) was not much different from that of late-onset sepsis (LOS; after 72 hours). Indeed, the relative proportion of each pathogen isolated on different days of life was similar (Appendix Figure 3).

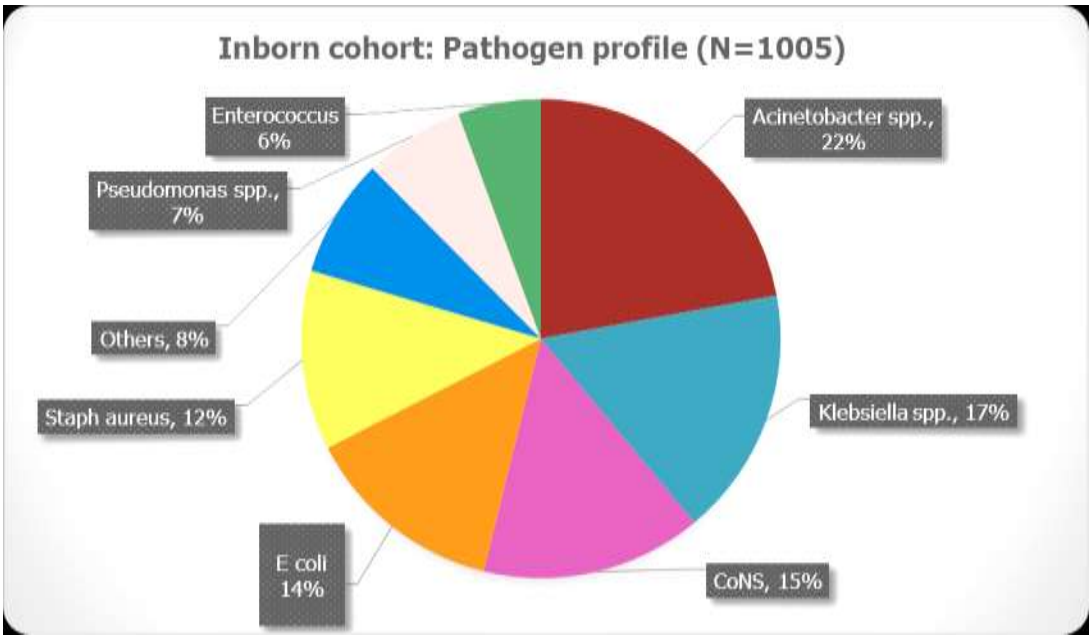

Figure 4: Pathogen profile in inborn cohort

- ***Antimicrobial resistance (AMR)***
  - Majority of the isolated pathogens exhibited high degree of AMR not only to commonly used antibiotics but also to 'reserve' antibiotics like extended spectrum cephalosporins and carbapenems (Figure 5 and Appendix Tables 7 and 8).
  - A high proportion of *Acinetobacter* spp. (81.5%), *Klebsiella* spp. (53.8%), and *E coli* (37.9%) were MDR. Colistin resistance was detected in seven (0.7%) Gram-negative isolates.
  - Among Gram-positive pathogens, methicillin resistance was detected in 60.7% of CoNS and 37.7% of *S aureus*. All the isolates of CoNS and *Staphylococcus aureus* were susceptible to vancomycin but about a quarter of enterococci isolates were resistant.
- ***Mortality outcomes***
  - Sepsis was the underlying cause of death in nearly a quarter (24·0%) of neonates.
  - The case fatality rate (CFR) of total and culture-positive sepsis was 25·6% and 47·6%, respectively (Table 3). The CFRs were not different between EOS (49·1% for culture-positive sepsis) and LOS (45.1%).
  - The attributable risks of mortality in culture negative sepsis, culture positive sepsis by 'multidrug-resistant' organisms, and culture positive sepsis by 'not multidrug-resistant' organisms were 88·6%, 97·3%, and 96·9%, respectively; the corresponding population attributable risks were 8·6%, 15·7%, and 12·0%, respectively (Appendix Table 9).
  - There was considerable variation in incidence, pathogen profile and AMR pattern across the sites, reflecting heterogeneity in case-mix and burden handled by the participating sites.
- ***Risk factors***
  - The significant risk factors for early and late onset sepsis on multivariate analysis are provided in Appendix (Appendix Tables 10 and 11); in general, the predictive abilities of risk factors were low for both EOS and LOS (AUC 0.72 and 0.84, respectively).

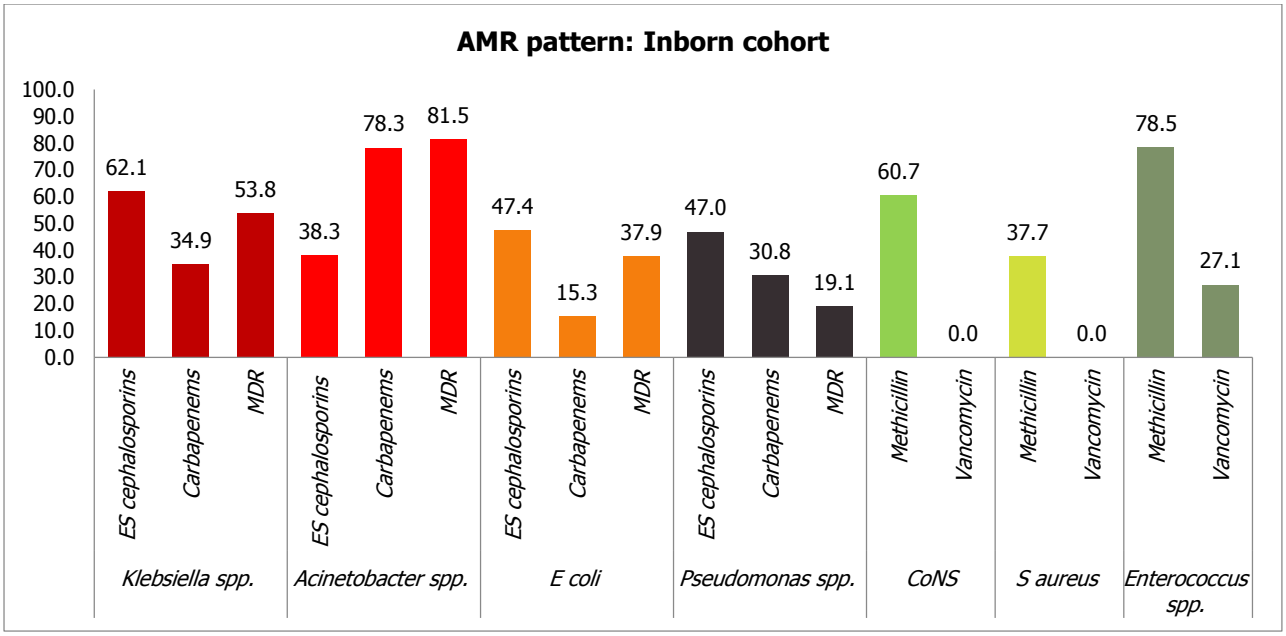

Figure 5: AMR pattern in common isolates

(CoNS, coagulase negative staphylococci; ES, extended spectrum; MDR, multi-drug resistant)

B) Outborn cohort

- ***Demographic data***
  - A total of 2588 of 2643 neonates admitted from July 2011 to February 2015 were enrolled (Study flow: Appendix Figure 4).
  - The mean birth weight and gestation were 2204 g and 35.4 weeks, respectively (Table 4). Over four-fifths of neonates were born at or after 32 weeks and had a birth weight of 1500 g or more. About two-thirds were boys (65%). Two-thirds were admitted within the first week of life – nearly a quarter within 24 h.
  - One-fifth of neonates was born at home – most were delivered by traditional birth attendants. Around one-third received unhygienic cord practices and prelacteal feeds (Table 4; Appendix Table 12).
  - Over a third (38.0%) were referred after being admitted at another health facility – mostly private hospitals – after a median (IQR) duration of stay of 2 (1 to 7) days. Most of them (83.8%) had received antibiotics for a median duration of 3 (1 to 6) days during prior hospitalization.

Table 4: Demographic characteristics of enrolled infants

Characteristics	Values (n =2588)
Birth weight, *g* (n= 2058)	2204±731
Gestational age, week (n=2583)	35.4±2.8
Male gender	1680 (64.9%)
Age at admission, days	5 (2-11)
Maternal details	
Fever within 7 days prior to delivery	211/2303 (9.2%)
Rupture of membranes (>18h)	397/2577 (15.4%)
Foul smelling liquor	53/2550 (2.1%)
Birth details	
Caesarean delivery	575/2580 (22.3%)

Home delivery	550/2588 (21.2%)
Did not cry at birth	643/2577 (24.9%)
Unhygienic cord practices	879/2544 (34.5%)
Previous hospitalization (n=984)	
Healthcare facility	
Primary/secondary level government hospital	56 (5.7%)
Tertiary level government hospital	198 (20.1%)
Private hospital	730 (74.2%)
Duration of stay, days	2 (1 to 7)
Antibiotic therapy	825/984 (83.4%)

Data expressed as no (%), median (IQR) or mean±SD

- ***Prevalence of sepsis***
  - Overall, 2280 episodes of sepsis were suspected in 2118 (81.8%) neonates, and 4365 cultures were processed (Table 5).
  - Sepsis was diagnosed in 1416 neonates (54.7%; 95% CI 52.8 to 56.6) and in 1458 episodes.
  - A total of 339 neonates (13.1%; 95% CI 11.8 to 14.5) had culture-positive sepsis. Meningitis, necrotizing enterocolitis, and systemic fungal infections were diagnosed in 7.0%, 4.4%, and 9 3.5% neonates, respectively.

Table 5: Outcomes of enrolled neonates (n=2588)

Variables	Results n (%)
Total sepsis	1416 (54.7%)
Culture positive sepsis	339 (13.1%)
Culture negative sepsis	1077 (41.6%)
Meningitis	180 (7.0%)
Necrotizing enterocolitis	113 (4.4%)
Systemic fungal infection	91 (3.5%)
Others*	13 (0.5%)
Case fatality rates, n (%)	
Total sepsis	151/1416 (10.7%)
Culture positive sepsis	78/339 (23.0%)
Culture negative	73/1077 (6.8%)
Meningitis	22/180 (12.2%)
Necrotizing enterocolitis	20/113 (17.7%)
Systemic fungal infection	20/90 (22.2%)

Data expressed as no (%)

- ***Profile of pathogens***
  - A total of 401 pathogens were isolated; bacterial pathogens accounted for about three-fourths (Appendix Figure 5). There was a predominance of Gram-negative isolates (52.9%), with common isolates being *Klebsiella pneumoniae* (12.5%), *Acinetobacter baumannii* (11.5%), *E. coli* (8.0%), and *Enterobacter cloacae* (5.7%).
  - The common Gram-positive pathogens were *Staphylococcus aureus* (4.7%), *S epidermidis* (4.2%), *S. hemolyticus* (3.2%), and *Enterococcus faecium* (3.0%).
  - A quarter of isolates were fungi, predominantly comprising of *Candida tropicalis* (5.0%), *C. albicans* (5.0%), and *C. parapsilosis* (4.5%).
- ***Antimicrobial resistance (AMR)***
  - Most bacterial isolates revealed high degree of AMR (Figure 6; Appendix Tables 14, 15 and16), even to "rescue" antibiotics. About 70% to 90% of *Klebsiella pneumoniae* and *Acinetobacter*

baumannii isolates were resistant to carbapenems and were MDR; carbapenem resistance was less common in *E. coli* (34.4%; Figure 6).

- High rates of methicillin resistance were noted in *S epidermidis* (88.2%), *S hemolyticus* (100%), and *S aureus* (31.6%). Vancomycin resistance was found in 41.7% of enterococci.

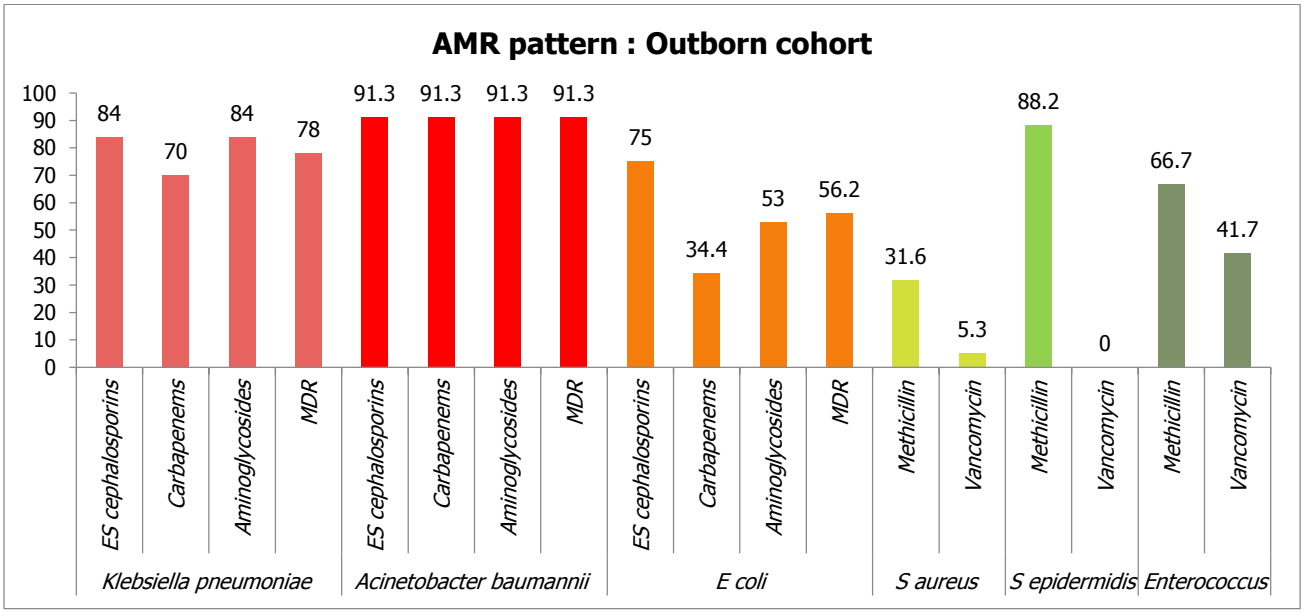

Figure 6: Antimicrobial resistance (AMR) in outborn cohort

(CoNS, coagulase negative staphylococci; ES, extended spectrum; MDR, multi-drug resistant)

- ***Systemic fungal infections***
  - Among the 90 neonates diagnosed with systemic fungal infections, two-thirds were born at or after 32 weeks of gestation and had a birth weight of 1500 g or more.
  - Almost all were diagnosed within 12 hours of admission to index hospital: median [IQR] of admission-to-detection interval 0 [0-1] h (Appendix Panel 2).
- ***Mortality outcomes***
  - Sepsis was the most common cause of death, accounting for two thirds of total deaths (153/243; 63.0%; 95% CI 56.6 to 69.0; Table 2).
  - The case fatality rates (CFR) of culture-positive and culture negative sepsis were 23.0% and 6.8%. Systemic fungal infections also showed comparable CFR (22.0%). Among the neonates with MDR sepsis, only half survived (74/131, 56.5%). %). Pathogen specific CFR is provided in Appendix (Appendix Table 13).

C) Molecular work

- ***Carbapenem resistant Klebsiella (CRK)***
  - Majority (n=105/106) were *Klebsiella pneumoniae*; one *Klebsiella oxytoca* was identified in the outborn site.
  - Among the genes responsible for carbapenem resistance, most (81.1%) CRK isolates were found to carry the novel member of metallo β- lactamases i.e. NDM-1 (Table 6 and Appendix Table 14). Other members of MBLs- VIM, and IMP genes- were not expressed, neither was KPC gene detected in any of the MHT positive strains.
  - The presence of NDM-1 gene was confirmed by DNA sequencing (99% to 100% BLAST identity match) (Accession no: Appendix Table 15).
- ***Carbapenem resistant Acinetobacter (CRA):***
  - Molecular work was completed for 294 strains – nearly all (98%) were confirmed as *Acinetobacter baumannii*, three strains (1%) were identified as *Acinetobacter baumannii - calcoaceticus* complex and one (0.05%) isolate was *Acinetobacter lwoffii*.

- Among the β-lactamase genes analyzed for carbapenem resistance, a quarter (n=70) of the isolates were NDM-1 positive while seven (3.5%) strains expressed another β-lactamase resistance gene- IMP (Table 6 and Appendix Table 19).
- Of the oxacillinase genes, OXA-51 gene was expressed in all the A. baumannii isolates, OXA-23 was expressed in the majority (87.7%), and OXA-58 in a few (10%) isolates; however, OXA-24 was not expressed in any isolate.
- DNA sequence results (BLAST) showed 98-99% identity match for the identified genes Accession no: Appendix Table 16).

Table 6: Genotyping for carbapenemase producers

Carbapenem resistant isolates	Processed for Molecular analysis	NDM-1	Other genes
Klebsiella spp. (CRK) (n=106/215)	100%	**86/106 (81.1%)**	0*
Acinetobacter spp. (CRA) (n= 317/386)	92% (294/317)#	**70 (24%)**	VIM= 0 IMP= 7 (2.4%) OXA-51= 293 (100%) OXA-58= 29 (10%) OXA-23= 258 (87.7%)

**screening for VIM, IMP and KPC genes- not amplified;*

 # 23 isolates from one site could not be revived

Key conclusions

- ***Inborn cohort***
  - There was a high incidence of sepsis in neonates admitted in NICUs; the case fatality rate was also high with sepsis alone accounting for nearly one-fourth of deaths
  - Majority of infections occurred early – nearly a quarter of culture positive sepsis episodes occurred within 24 h and two thirds within 72 h.
  - *Acinetobacter* spp. emerged as the predominant causative organism; this is in contrast to the results of NNPD (2002-03) in which *Klebsiella* spp. (32%) was reported to be the leading pathogen and the proportion of *Acinetobacter* spp. was only 3%.
  - Most pathogens showed an alarming degree of antimicrobial resistance.
- ***Outborn cohort***
  - The prevalence of sepsis was very high; it was also the foremost cause of in-hospital mortality
  - There was a predominance of invasive fungal infections – about a quarter of neonates with culture positive sepsis had systemic fungal infections
  - An alarmingly high degree of AMR and an unexpectedly high level of AMR was observed
- ***Molecular epidemiology***
  - Molecular analysis of carbapenem resistance genes revealed a very high prevalence of the novel metallo-beta-lactamase gene, NDM-1 in carbapenem resistant *Klebsiella* and *Acinetobacter* isolates.

Future plans

- Manuscript on **sepsis profile** in **intramural cohort** is **accepted** for publication in ***Lancet Global Health***; manuscript on profile in extramural cohort under review in *PLoS One*
- Plan to submit the manuscripts on molecular epidemiology of carbapenem resistant Klebsiella and Acinetobacter in *Scientific Reports* and *Clinical Infectious Disease,* respectively by July 31, 2016, and the manuscript on risk factors of sepsis in *Pediatrics* by August 15, 2016
- As a follow-up of the sepsis registry, we submitted a proposal to DBT, the aim of which is to understand the biology of neonatal sepsis; the major objectives are to
  - evaluate the diagnostic utility of procalcitonin (PCT) and 10 other novel/non-validated biomarkers in neonatal sepsis
  - identify the novel host and pathogen specific bio-signatures of neonatal sepsis using the "omics" technology (in collaboration with THSTI, Gurgaon & NCBS, Bangalore)

    The project was funded by DBT (1.84 crores) in May 2015; the study is being conducted in two sites (Safdarjung hospital & AIIMS)
- Collaborate with National Institute of Health (NIH) – Antibiotic Resistance Leadership Group (ARLG) and ICMR & DBT, Govt.of India in a collaborative project on antimicrobial resistance, the specific aims of which are to
  - undertake molecular epidemiology of multidrug resistant Acinetobacter infection in neonates in India and to
  - validate and test new diagnostic technologies and sharing of clinical protocols to, in part, enhance clinical research
- Submit the LoI "Role of sepsis screen and procalcitonin in early onset neonatal sepsis – a diagnostic RCT" to ICMR/DBT in the recently announced Call for Proposals in Neonatal Sepsis

Project 2: Effect of stepwise interventions on hand hygiene compliance rates among healthcare providers: A before-and-after study

Deviations from original protocol

Project 2 was initially sanctioned as a multi-centric RCT to evaluate the efficacy and safety of whole body application of chlorhexidine soon after birth in preterm very low birth weight (VLBW) neonates. When the study was about to be launched after obtaining clearance from the Institutional Ethics Committees and after developing indigenously prepared chlorhexidine wipes, the new regulations regarding drug trials emerged which made prior approval from DCGI mandatory for any drug trial. In the meantime, the investigators systematically reviewed the evidence on the safety and efficacy of whole body chlorhexidine application – high quality evidence from randomized trials showed no significant benefits in either the risk of mortality or the incidence of sepsis; on the other hand, there was paucity of evidence on the safety of chlorhexidine application, particularly in preterm neonates. The updated evidence was presented in the Scientific Advisory Committee (SAC) meeting and the issues were discussed at length. The experts of the SAC concurred with the concerns raised by the Investigators and recommended not to initiate the trial. Instead, the SAC recommended initiating studies on Quality Improvement (QI) in the domain of neonatal sepsis – initially as a pilot study in one of the study sites and then to replicate the same at the other sites, if possible.

Objectives

Primary

To compare the hand hygiene compliance rates among staff nurses and residents in a level-3 NICU before and after implementation of a stepwise improvement package comprising 4 strategies namely, standard learning, intensive learning, video monitoring, and video monitoring with feedback

Secondary

i. To evaluate the incremental improvement in hand hygiene compliance rates before and after the introduction of *each* individual strategy

ii. To evaluate the effect of the intervention on compliance for each of the WHO five moments of hand hygiene

Outcome measure

Hand hygiene compliance rate; compliance was defined as the number of positive actions/number of opportunities as per the WHO guidelines on hand hygiene

Methods

- The study was conducted in a level-3 NICU (AIIMS), after a pilot/training period of one month.
- Resident doctors and bedside nurses involved in routine care of neonates in NICU were enrolled in the study.
- The study was done in five phases – the first phase (Phase 0) being the baseline period and the next four being the period during and after introduction of the four strategies of intervention, namely (1) standard learning; (2) intensive learning and motivational/behavioral change; (3) closed circuit television (CCTV) monitoring; and (4) CCTV monitoring with individual feedback. The individual strategies were introduced sequentially, with each phase lasting for about four weeks (Figure 7).

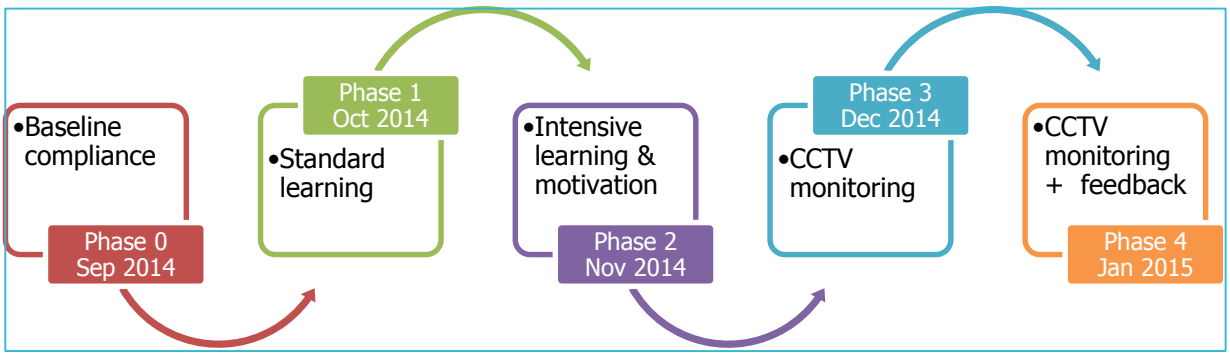

Figure 7 Study plan

- A brief description of the four strategies is as follows:
  - ***Strategy 1: Standard learning***
    - Included display of posters and placards, video demonstration, and distribution of self-learning module
    - Posters on WHO five moments and how to do hand hygiene were placed at key locations including the washbasins, NICU beds, and as screen savers in the NICU computers.
    - Nurses and residents were shown the standard teaching module on hand hygiene once – in 30 min sessions – over 4 weeks. Self-learning videos and PowerPoint presentations on the importance, WHO five moments and technique, and 'Hand-outs' on hand hygiene were distributed.
    - Availability of adequate supplies of liquid soap, hand rub, and paper napkins ensured.
  - ***Strategy 2: Intensive learning and motivation***
    - Included daily classes and practical demonstrations, behavioral modification of providers by the faculty, and periodic certificates of appreciation.
    - Daily 30-min classes held in batches of 3-5 nurses on the importance and technique of hand hygiene; twice weekly 30-min classes for 1-2 residents.
    - The faculty re-emphasized the importance of hand hygiene, motivated the providers on a regular basis, and acted as role-model in strictly following the hand hygiene protocol
    - An independent nurse educator identified five 'best' nurses/residents who strictly complied with hand hygiene protocol every week; they received token gifts and certificates of appreciation from the faculty.
    - Daily reminders by SMS/WhatsApp on the importance of hand hygiene and hand hygiene protocols sent to the healthcare providers
  - ***Strategy 3: Video monitoring by closed circuit television (CCTV) camera***
    - CCTV cameras were installed in NICU – 5 cameras including one high-definition (HD) camera with zooming facility inside the NICU; one camera near the washbasin at NICU entry
  - ***Strategy 4: Video monitoring and feedback***
    - Output from the CCTV camera was viewed at random in all three shifts of the day by the principal investigator (PI)
    - Personal feedback was sent by SMS to the healthcare provider who had missed any of the steps of hand hygiene.
    - A positive message was also sent to those who followed all the steps of hand hygiene.

Outcome measurement

- Compliance was measured as the number of positive actions/number of opportunities.
- Four research nurses (RN) were trained for recording hand hygiene opportunities and their corresponding actions according to the WHO five moments of hand hygiene; their observations were independently validated by the PI before the study began.
- RN observed the hand hygiene opportunities and actions of the providers caring for neonates at randomly selected beds at randomly selected times of each of the three duty shifts. A computer generated random number sequence (kept in sealed envelopes in NICU) was used to identify the bed as well as the start time.
- For each observation period in a shift, a maximum of 3 healthcare providers working at the selected beds were observed for around 45 min each. The RN identified the opportunities for hand hygiene and followed the complete sequence of hand hygiene of a particular healthcare provider for a maximum of 8 opportunities.
- RN recorded either a positive hand hygiene action or missed action on the standard form adapted from the WHO guidelines.

Key results

- The study was conducted from September 2014 to January 2015. A total of 55 healthcare providers (40 bedside nurses and 15 residents) were eligible for observation during the study period (Study flow: Appendix Figure 6).
- A total of 7134 opportunities were observed during the study period. The mean (SD) duration of observation was 56 (22) minutes.

- *Baseline (Phase 0):* Of the 1002 opportunities observed, the healthcare workers were compliant with all steps of hand hygiene on 619 occasions (compliance of 61.8%; Table 7).
- *Compliance after intervention(s):* The compliance rate showed a significant improvement to 77% (relative risk difference 25%; 95% CI 18% to 32%) after implementation of all four strategies (Table 2). Both the nurses and residents showed a similar degree of improvement.
- *Compliance for the WHO five moments*
  - The compliance rates of the WHO five moments showed significant improvement in moments "before touching the patient" (moment 1; from 66.3% at baseline to 79.3%), "after touching the patient" (moment 4; 61.3% to 80.1%), and most remarkably, "after touching patient surroundings" (moment 5; from 27.7% to 67.4%).
  - Hand hygiene compliance was better for the WHO "before" moments as compared to "after" moments of hand hygiene both at baseline and at the end of phase 4.

Table 7 Hand hygiene compliance before and after all 4 phases of intervention

Compliance	Baseline (Phase 0)	After 4 steps of interventions (End of Phase 4)	% Change in compliance from baseline	
			Absolute risk difference (95% CI)	***Relative risk difference (95% CI)***
Overall	619/1002 (61.8%)	835/1085 (77.0%)	15.2% (11.3% to 19.1%)	25% (18% to 32%)
Category of healthcare providers				
Resident doctors	93/154 (60.4%)	52/70 (74.3%)	13.9% (1.1% to 26.7%)	23% (2% to 49%)
Bedside nurses	526/848 (62.0%)	783/1015 (77.1%)	15.1% (11.0% to 19.3%)	24% (17% to 32%)
WHO five moments*				
Moment 1	66.3%	79.3%	13.0% (6.5% to 19.5%)	19.6% (9.1% to 31.1%)
Moment 2	85.5%	81.5%	-4.0% (-12.0% to 3.9%)	0.95% (0.86% to 4.9%)
Moment 3	71.3%	79.3%	6.0% (-11.4% to 23.3%)	8.2% (0.86% to 35.3%)
Moment 4	61.3%	80.1%	18.8% (11.3% to 26.5%)	30.7% (16.7% to 46.5%)
Moment 5	27.7%	67.4%	39.8% (31.3% to 48.2%)	143.5% (91.2% to 210.3%)

**Moment 1: Before touching a patient; 2: Before clean/aseptic procedure; 3: After body fluid exposure risk; 4: After touching a patient; 5: After touching patient surroundings*

- *Incremental effect of each individual strategy*
  - The compliance rates measured sequentially during the four phases of intervention – phases 1 to 4 – were 74.9%, 77.5%, 70.1%, and 77.0%, respectively (Table 8).
  - The relative risk difference between the consecutive phases were 21% (phase 0 to phase 1), 4% (phase 1 to phase 2), -10% (phase 2 to phase 3), and 10% (phase 3 to phase 4).
  - An incremental effect was observed after standard learning and after CCTV monitoring with individual feedback. CCTV monitoring alone showed a significant decrease in the compliance rates when compared to phase 2 (intensive learning).

Table 8 Hand hygiene compliance during each phase of intervention

	Results	Baseline (Phase 0)	Standard learning (Phase 1)	Intensive learning & motivation (Phase 2)	CCTV (Phase 3)	CCTV and feedback (Phase 4)
Overall	Compliance	619/1002 (61.8%)	1041/1390 (74.9%)	1460/1884 (77.5%)	1243/1773 (70.1%)	835/1085 (77.0%)
	Incremental effect (95% CI)	-	21% (15% to 28%)	4% (0% to 8%)	-10% (-13% to -6%)	10% (5% to 15%)

	P	-	< 0.001	0.08	< 0.001	< 0.001
Nurses	Compliance	526/848 (62.0%)	965/1288 (74.9%)	1192/1560 (76.4%)	1180/1692 (69.7%)	783/1015 (77.1%)
	Incremental effect (95% CI)	-	21% (14% to 28%)	2% (-2% to 6%)	-9% (-12% to -5%)	11% (6% to 16%)
Residents	Compliance	93/154 (60.4%)	76/102 (74.5%)	268/324 (82.7%)	63/81 (77.8%)	52/70 (74.3%)
	Incremental effect (95% CI)	-	23% (4% to 46%)	11% (-2% to 26%)	-6% (-17% to 7%)	23% (4% to 46%)

Conclusions

- Hand hygiene compliance was relatively high at baseline among nurses and residents in NICU; it improved significantly at the end of the stepwise improvement package
- An incremental effect on compliance was observed with standard learning and CCTV monitoring with individual feedback; CCTV monitoring alone didn't improve the compliance rates much

Future plans

- Manuscript is ready for submission in *Pediatrics* journal
- Submit the following proposals for funding to National/International agencies:
  - Quality Improvement for reducing the incidence of HAIs in major hospitals of Delhi
  - Developing a QI Collaborative Network for sepsis in SNCUs with bench marking and support by level-3 neonatal units (including AIIMS, MAMC, PGI, etc.)

ADDITIONAL PROJECTS UNDER THE THEME OF 'NEONATAL SEPSIS'

Metabolomic profile of healthy neonates

(PhD Thesis of Dr Suman Chaurasia, Scientist-3, supported by ICMR-CAR)

Rationale: Metabolomics is the youngest of the 'omics' sciences, and is yet to make a significant strides in neonatology. Increasingly researchers have used the metabolomic approach in the fields of congenital malformations, perinatal asphyxia, fetal origins of disease, inborn errors of metabolism, respiratory, cardiovascular and nutritional disorders. Remarkable progress has been made towards newer understanding and earlier diagnosis in these areas. However, there is paucity of data regarding normalcy in healthy infants.

Objectives

Primary: To identify the urinary metabolome of healthy term male infants of gestational age 39-40 weeks (full term) and postnatal age 24-72 hours

Secondary: To determine the influence/contribution of breast-milk on urinary metabolomic profiles in healthy term male infants of gestational age 39-40 weeks and postnatal age 24-72 hours

Potential outcomes and translational potential

Defining the normalcy is the fundamental step to determine optimal standard of health, which enables comparison with disease conditions for newer learning. With the larger goal to facilitate future metabolomic research in neonatal illnesses as sepsis, respiratory distress syndrome or perinatal asphyxia, this study in collaboration with International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi is an exploratory study to be pursued as PhD thesis.

Present status

In the study, interim analysis of one-thirds of the subject was performed recently and the on-going recruitment is now nearly complete.

THEME 2: FEEDING AND NUTRITION OF LBW INFANTS

Project 3: Vitamin D status and need for supplementation among LBW infants

Deviations from original protocol

The initial proposal was to do a two-phase study to answer the following research questions:

Phase 1:

> *What is the incidence of vitamin D deficiency (VDD; <20 ng/ml or 50nmol/L) in infants born low birth weight during infancy (measured at 3, 6 and 12 months of age) and whether the vitamin D deficiency profile different in preterm LBW infants compared to term LBW (IUGR) infants?*

Phase 2:

> *What is the optimum dose of supplementation of vitamin D- 400 IU per day versus 800 IU per day for one year- to prevent this deficiency state (as measured radiographically by bone mineralization at 6 and 12 months of age)?*

The objective of the first phase overlapped with another contemporary ICMR-funded multisite study of the Division, which estimated the vitamin D levels at birth and in early infancy among low and normal birth weight infants (*Agarwal R, Virmani D, et al. Vitamin D Status of Low Birth Weight Infants in Delhi: A comparative study. J Trop Pediatr 2012;58:446-50).*

Thus, as part of CAR project, we focused on the next phase evaluating the two regimens of vitamin D (400 vs. 800 IU among very preterm infants.

Hypothesis

Daily supplementation with 800 IU of vitamin D3 would reduce the prevalence of vitamin D insufficiency by 50% at 40 weeks postmenstrual age in preterm infants born at 28 to 34 weeks of gestation when compared to daily supplementation with 400 IU)

Objectives

Primary

To compare the prevalence of VDD (25(OH)D_3<20 ng/mL) following daily supplementation with 400 IU vs. 800 IU of oral vitamin D_3 at 40 weeks postmenstrual age in preterm infants born at 28 to 34 weeks of gestation.

Secondary

To compare the prevalence of VDI, bone mineral content (BMC), and bone mineral density (BMD) at 3 months corrected age (3mCA).

Outcome measures: proportions of infants with VDD, and those with VDI, bone mineralization as measured in terms of BMC and BMD (determined by dual energy X-ray absorptiometry (DEXA)).

Methods

In this double blind RCT, eligible preterm neonates LBW infants (free from congenital malformations and bone dysplasias) at birth and were started routine vitamin D supplementation starting by 2 weeks and continued through 3 months of age. The enrolled participants were followed up monthly to ensure compliance with treatment and look for clinical signs of rickets. Serum calcium, phosphate, alkaline phosphatase, vitamin 25(OH)D, and parathyroid hormone (PTH) were estimated at 3 months' CA; bone mineral density (BMD) was assessed using DEXA.

We calculated our sample size based on the assumed prevalence of VDD of 75% in 400 IU group, 90% power, and an alpha error of 0.05, we needed to enroll 40 babies per group to detect a 50% relative reduction in the vitamin D insufficiency following supplementation of 800 IU/day.

Results

- The prevalence of VDD in '800 IU group' was significantly lower than '400 IU group' at 40 weeks (38.1% vs 66.7%; RR: 0.57; 95% CI: 0.37–0.88) and at 3 months' CA (12.5% vs 35%; RR: 0.36; 95% CI: 0.14–0.90; Table 9 and Figure 8). The bone mineral content (BMC) and bone mineral density at 3 months were also not different between the groups.

- One infant (2.4%) in the '800 IU group' had vitamin D excess (100 to 150 ng/mL).

Table 9: Primary and key secondary outcomes

Variable	800 IU group (N=42)*	400 IU group (N=45)*	Relative risk (95% CI)	p
At 40±2 weeks				
Vitamin D deficiency (<20 ng/mL)	16 (38)	30 (67)	0.57 (0.37to 0.88)	0.008
Vitamin D severe deficiency (<5ng/mL)	0	2 (4.4)	-	0.50
At 3 months corrected age				
Vitamin D deficiency (<20 ng/mL)	5 (12)	14 (35)	0.36 (0.14to 0.90)	0.03
Vitamin D severe deficiency (<5 ng/mL)	0	1 (2.5)	-	1.0
Bone mineral content (g)	79.6 (16.8)	84.7 (20.7)	-5.1 (-14.1, 4.0)	0.27
Bone mineral density (g/cm^2)	0.152 (0.019)	0.158 (0.021)	-0.005 (-0.02 to 0.004)	0.26

**N=40 at 3 months corrected age in both the groups*

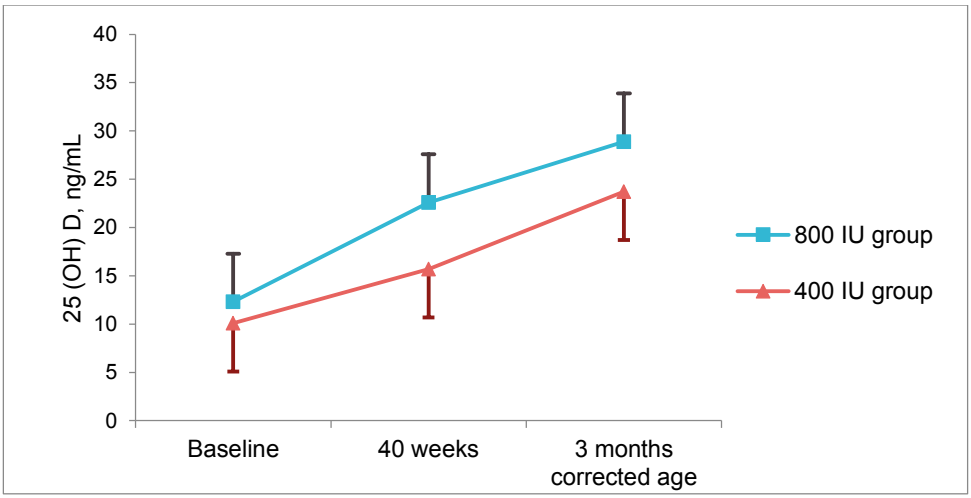

Figure 8: Vitamin D levels at different time points

Conclusions: Daily supplementation with 800 IU of vitamin D reduces the prevalence of vitamin D insufficiency at 40 weeks and at 3 months' corrected age in preterm infants without having any improvement in bone mineralization. However, there is a possibility that this dose may result in vitamin D excess occasionally.

Publications/achievements

- Manuscript got published Pediatrics Journal (Natarajan CK et al. 2014;133:e628–34; Impact Factor: 5.5)
- Study won NNF Gold Medal for the 'Best research paper' at NNF Annual Meeting 2012; American Academy of Pediatrics (AAP) had chosen it for public release during PAS meeting in May 2013

ADDITIONAL PROJECTS ON VITAMIN D SUPPLEMENTATION IN NEWBORNS

1. Prevalence of vitamin D deficiency at 12 to 16 weeks of age in term healthy Indian infants receiving supplementation of 400 IU of vitamin D from birth: interventional case series.

Background: American Academy of Pediatrics (AAP), Institute of Medicine, USA and Indian Academy of Pediatrics recommend daily supplementation of vitamin D in a dose of 400 IU to breastfed and partially breastfed infants. Given the high prevalence of VDD, this recommendation of 400 IU/day by international bodies like AAP and IAP may not be sufficient to ensure optimum levels of vitamin D in Indian neonates.

We sought to evaluate the prevalence of VDD at 3 months in term healthy infants supplemented with daily Vitamin D (400 IU) from birth.

Primary objective: To determine the prevalence of vitamin D deficiency (defined as 25(OH)D < 15 ng/mL) at 12- 16 weeks in term healthy breastfed infants supplemented daily from birth with 400 IU of vitamin D (Table 15).

Secondary objectives: (1) To determine prevalence of vitamin D deficiency at birth in term healthy neonates; (2) To ascertain the prevalence of metabolic and clinical rickets at 12- 16 weeks in infants supplemented from birth with 400 IU of vitamin D

Methods

In this prospective interventional study, we enrolled 111 term infants at birth and started on oral vitamin D supplementation in a dose of 400 IU. Primary outcome was prevalence of VDD (levels <20 ng/ mL) and vitamin D insufficiency (VDI, vitamin D levels 20-29 ng/mL) at 14 ± 2 weeks of age. Secondary outcomes were prevalence of a) VDD or VDI at birth, b) metabolic rickets (elevated serum alkaline phosphatase> 420 U/L) at 14 ± 2 weeks and c) clinical rickets at 14 ± 2 weeks.

Results

- The mean gestation and birth weight of enrolled neonates were 38.0 wk and 3059±329 g, respectively. All infants were started on 400 IU of vitamin D supplementation within a week of birth. Only 3 infants (3.2%) missed > 20% of doses.
- At birth, 89 neonates (83.2%; 95% CI 74.7 to 89.7%) were deficient and an additional 13 (12.2%; 6.6 to 19.9%) were insufficient; the mean serum vitamin D was 12.5 ± 7.7 ng/mL.
- At 14±2 weeks' age, 47 infants (52.2%; 95% CI: 41.4 to 62.8%) had VDD, while an additional 35 (38.9%; 28.8 to 49.7%) had VDI. The mean serum vitamin D was 19.4 ± 7.3 ng/mL.
- No infant developed clinical signs of vitamin D toxicity.
- Clinical rickets was noted in 4 infants (4.5%); all of them had VDD with elevated alkaline phosphatase and normal levels of serum calcium and phosphorus.

Interpretation: The prevalence of VDD and VDI continues to be high at 14 weeks of age in term healthy infants in India despite daily oral dose of 400 IU of vitamin D3, possibly indicating it as suboptimal dose.

Publications: The manuscript is being submitted for publication in *Pediatrics* Journal.

2. Study on efficacy of daily supplementation of 800 IU vitamin D on vitamin D status at six months of age in term healthy infants

Background

In view of the finding of previous study which indicated suboptimal dose for maintaining normal vitamin D levels in Indian neonates, we planned this study the effect of supplementation of 800 IU vitamin D on vitamin D sufficiency status in term healthy breastfed infants.

Primary objective: To determine the prevalence of VDD (25(OH)D <20 ng/mL) at 6 months in term healthy breastfed infants supplemented with 800 IU of vitamin D per day starting from birth

Secondary objective: To determine the

1. Proportion of vitamin D excess or toxicity in the first 6 months of age
2. Postnatal age at which plateau effect of vitamin D supplementation is achieved by comparing vitamin D levels at 6, 10 and 14 weeks and 6 months of age0) and 14 weeks & 6 months (cohort 3; n=20)

Methods: In this prospective interventional study, we supplemented 800 IU/day of vitamin D in term healthy infants (n=70) starting within 48 hours of birth and continued until 6 months of age. Serum 25(OH)D was measured at birth and 6 months for all infants. In addition, $25(OH)D_3$ was measured in subsets of 23, at 6, 10 and 14 weeks of age. The primary outcome was prevalence of VDI (defined as serum $25(OH)D_3$ level <30 ng/mL) at 6 months of age.

Results

- Nearly 83% (58/70) infants were followed-up until six months of age.
- The median (ng/mL; IQR) serum 25(OH)D at birth and 6 months of age was 10 (5-14) and 37 (29-55), respectively.
- Prevalence of VDI at birth was 98.5% (68/69), which reduced to 27.6% (16/58) at 6 months of age.

- However, four infants (6.9%, 95% CI 1.9% - 16.7%) developed vitamin D excess (25(OH)D 100-150 ng/mL) requiring reduction of the dose of supplementation. No infant developed vitamin D toxicity (serum 25(OH)D >150 ng/mL).
- The maximum concentration was attained at 6 weeks of age (mean 52 ng/mL) with somewhat steady values thereafter.

Conclusions

- Daily supplementation of 800 IU of vitamin D resulted in vitamin D sufficiency in most term healthy infants at 6 months of age.
- However, in view of potential risk of toxicity in some infants, this regime cannot be universally recommended in all infants.
- Alternative approaches including correction of vitamin D deficiency at birth by appropriate mega dose followed by routine supplementation of a lower dose (400-600 IU) should be explored as a strategy to achieve vitamin D sufficiency in these infants.

Present status

The study has been completed and the manuscript is being submitted to *Pediatrics* journal.

ADDITIONAL PROJECTS ON FEEDING AND NUTRITION OF LBW NEONATES

Complementary feeding at four versus six months of age in preterm infants: a randomized, multicenter trial

(PhD thesis of Dr Shuchita Gupta, Scientist-3, supported by ICMR-CAR)

Rationale: The current guidelines for exclusive breastfeeding till 6 months followed by complementary feeding meant for term healthy infants cannot be extrapolated to preterm infants as their nutrient requirements are likely to be higher, and there is no evidence to suggest till what time breast or formula milk alone would be able to meet their nutrient requirements in early infancy.

Research question: In preterm infants born before 34 weeks of gestation, does initiation of complementary feeding at 4 months compared to 6 months of corrected age (CA) improve the weight for age (WFA) z score at 12 months of CA?

Outcomes

- The primary outcome was growth in terms of weight for age z score at 12 months of CA.
- Secondary outcomes were body composition and bone mineral density and content measured by dual energy absorptiometry (DXA), iron status measured by serum ferritin, neurodevelopment measured by Developmental Assessment Scales for Indian Infants (DASII-2), morbidities requiring hospitalization during the study period and markers of metabolic syndrome like insulin resistance (HOMA-IR), blood pressure and lipid profile at 12 months of CA.

Methodology

- All intramural live births admitted to the NICUs at AIIMS, Safdarjung and Kasturba hospitals were tracked and infants meeting inclusion criteria were identified. These infants were kept in follow up and reassessed for eligibility at 4 months (17 weeks) of CA, and enrolled in the study following informed consent.
- They were randomized at enrolment into two groups: 4-month group, in which infants were started on complementary feeding at 4 months of CA, and 6-month group, in which infants were continued ion milk feeding and started at CF at 6 months of CA.
- Follow up telephonic calls were made on day 2, 7, 14 and 21 at 4 and 6 months of CA in both the groups. The infants were then followed up at 5,6,7,9 and 12 months of CA and anthropometric parameters, diet and morbidity records maintained at these visits. The study ended at 12 months of CA, when blood pressure, lipid profile, insulin sensitivity and DXA were done along with growth and neurodevelopment.
- A total of 403 infants were enrolled and randomized in the study between March 2013 and April 2015: 206 to 4-month and 197 to 6-month group. There were 4 deaths in 4-month and 2 deaths in the 6-month group. A total of 18 infants in 4-month and 4 infants in the 6-month group were lost to follow up at 12 months of CA.

- There was no difference in the primary outcome between the two groups: WAZ12 (mean±SD) being 1.6±1.2 in the 4-month vs. 1.6±1.3 in the 6-month group (mean difference 0.005, 95% CI -0.24 to 0.25; p=0.96; Table 10).
- There were more episodes of hospitalization in the 4-month compared to the 6-month group: Episodes per baby-month 0.02 vs. 0.01 (incidence rate ratio 1.8, 95% CI 1.0 to 3.3).
- Besides this, there was no difference in any other secondary outcomes like body composition, bone mineral content and density, lipid profile, HOMA-IR and BP between the two groups.
- However, a notable finding was that serum ferritin at 12 months of CA was quite low in both the groups (median serum ferritin: 5.4 µg/dL in 4-month vs. 5.7 µg/dL in 6-month group; p=0.73). Almost two-third infants in both groups (74.4% in 4-month and 72.8% in the 6-month group) had significantly depleted iron stores (serum ferritin <12 µg/dL).

Table 10. Primary and secondary outcomes

	4-month	6-month	Mean difference / RR (95% CI)	P
Primary outcome				
Weight for age z score	-1·6±1·2, n=184	-1·6±1·3, n=189	0·005 (-0·24 to 0·25)	0·96
Secondary outcomes				
Death	4/203*	2/197	1.9 (0.4 to 10.5)	0·44
Hospitalization				
Infants, n (%)	34/184 (18.1)	18/192 (9.4)	1.9 (1.1 to 3.3)	0·01
Episodes per infant-month	39/1548 (0·025)	23/1599 (0·014)	1.8 (1.0 to 3.1)#	0·03
Neurodevelopment				
$MoDQ_{50}$<70	27 (14·8), n=182	22 (12·0), n=184	1.2 (0.7 to 2.1)	0·55
$MeDQ_{50}$<70	12 (6·6%), n=181	12 (6·6%), n=182	1.0 (0.5 to 2.2)	0·71
Body composition				
Fat mass (g)	2056±714, n=134	2128±762, n=135	-72 (-250 to 105)	0·42
Lean + BMC mass (g)	6182±805, n=134	6265±922, n=135	-84 (-292 to 124)	0·43
Total mass (g)	8234±1129, n=134	8427±1208, n=135	-193 (-474 to 88)	0·18
Percent fat (%)	24·5±6·8, n=134	25·3±6·7, n=135	-0·8 (-2·4 to 0·8)	0·33
Bone mineral content (g)	186·0±35·7, n=135	191·8±32·9, n=135	-5·7 (-13·9 to 2·5)	0·17
Bone mineral density (g/cm^2)	0·25±0·03, n=135	0·25±0·03, n=135	-0·001 (-0·008 to 0·006)	0·77
HOMA-IR	0·4 (0·3-0·7), n=153	0·4 (0·2-0·7), n=166	-	0·36
Serum ferritin <12 µg/dL, n(%)	119/160 (74·4)	126/173 (72·8)	1.0 (0.9 to 1.2)	0·75

**Outcome of death was known for all enrolled infants except for one infant in the 4-month group; #Incidence rate ratio; Data are mean±SD, n (%), or median (IQR); Abbreviations- $MoDQ_{50}$-Motor developmental quotient- 50th centile, $MeDQ_{50}$-Mental developmental quotient - 50th centile, HOMA-IR- Homeostatic Model Assessment for Insulin Resistance*

Conclusions

- In infants <34 weeks of gestation, initiation of CF at four compared to six months of CA does not result in any difference in WAZ12 at 12 months of CA.
- However, the risk of hospitalization increases among infants in the 4-month group. There is no difference in any other growth parameters, body composition, bone mineral content and density, lipid profile, insulin resistance measure (HOMA-IR) and BP between the two groups. Iron stores are significantly depleted in both groups despite iron supplementation.
- It therefore implies that in infants less than 34 weeks of gestation, initiation of CF at six months of CA is preferable to four months of CA.

Publications/achievements

- The study is completed and been submitted for publication to NEJM

THEME 3: NEURODEVELOPMENT FOLLOW-UP

Project 4: Neurocognitive outcomes of high-risk infants

Deviations from the original protocol

In the original proposal, we proposed a sample size involving 500 LBW infants, 50 term infants with perinatal asphyxia and a similar number of control infants (total 1100). Later, due to logistic difficulty and to take equal representation of LBW infants, we planned to enroll 600 LBW (250 very preterm and 250 late preterm, and 100* term SGA infants), and 50 asphyxiated (or whatever number was available at AIIMS and 250 term AGA infants as controls.

Subsequently, as AIIMS has a low incidence of perinatal asphyxia, after enrolling 17 babies in this group, we discontinued enrolment in this group.

Objectives

- To determine neuro-cognitive outcomes at 18 months of high-risk neonates namely, preterm low birth weight (LBW), term LBW, and those with perinatal asphyxia
- To develop a systematic protocol driven approach for facility-based follow up of high-risk neonates

Methods

- This was a prospective cohort study conducted at the All India Institute of Medical Sciences (AIIMS), Delhi, India from 13th September 2011 to 14th November 2015; ethical clearance was obtained from the Institutional Ethics Committee.
- ***Participants:*** Five cohorts of eligible neonates were enrolled simultaneously at birth:
  - Very preterm (gestation ≤32 weeks and/or birth weight ≤1500 g)
  - Late preterm (gestation 34-36 weeks)
  - Term small-for-gestational age (SGA; birth weight <10th centile for gestational age according to AIIMS growth charts)
  - Term neonates with perinatal asphyxia (positive pressure ventilation for >60 seconds at birth) and
  - A 'control' comparator cohort of term healthy infants (appropriate for gestational age and not requiring NICU stay any time during birth hospitalization)
- Neonates with major congenital malformations, genetic or chromosomal abnormality, and those diagnosed to have congenital hypothyroidism, congenital adrenal hyperplasia or inborn error of metabolism during birth hospitalization were excluded from the study.
- Primary outcome was major disability defined as presence of cerebral palsy or developmental delay (DMeQ or DMoQ<70 using DASII) or hearing impairment requiring hearing aid or vision impairment (visual acuity <3/60 in the best eye, assessed using Cardiff acuity cards) or epilepsy (one or more provoked seizures at 18 months of age).
- The secondary objectives were to determine growth outcomes in terms of z-scores for weight, length, head circumference and weight for length, calculated using corrected age as per the WHO-MGRS growth standards,i through 18 months of age.
- Gestation was assessed using date of last menstrual period (LMP) and corroborated with first trimester ultrasound. The Expanded New Ballard Scoring (ENBS) was also performed within 48-72 hours of birth. Gestation by date of LMP was taken if there was a discrepancy of <2 weeks between LMP/USG and Ballard, else gestation assessed by Ballard was taken as final.
- Fetal growth category was assessed using AIIMS intrauterine growth charts, categorized as small-for-gestational age (SGA; BW<10th centile for GA), AGA (as above), or large-for-gestational age (LGA; BW >90th centile for GA).
- All live-births during the study period were tracked and eligible neonates identified at birth. Eligible infants were visited daily by social workers for tracking morbidities. Once they were stable and shifted to mother's side, they were enrolled following written informed consent.

- Anthropometry of all eligible infants was taken within 24 hours of birth by trained research team, and repeated at discharge for enrolled babies. For very preterm infants or those eligible infants requiring NICU admission, anthropometry taken as soon as the baby was clinically stable (in such cases, anthropometry at birth was recorded from neonatal case records).
- ***Follow-up schedule***: Infants were followed-up prospectively until 18 months of corrected age. Formal neurodevelopmental evaluation was done at 3 months of age (corrected age, if applicable) for preterm LBW and asphyxiated infants and then at 18 months of age. Ophthalmological evaluation was done at 9 months of age. Growth was monitored at 3 monthly intervals for very preterm and term SGA infants. A reminder call 1 week prior to the scheduled formal assessments at 3, 6, 9 12, and 18 months of corrected age was made and all other appointments were arranged accordingly.
- Additionally, an MRI of the brain and simultaneous cranial USG were done for very preterm infants of consenting parents at 40 weeks of corrected age (term equivalent age), with a maximum allowed delay of 6 weeks.
- Appropriate care in terms of early stimulation and rehabilitative services as required was provided to all infants.
- ***Outcome assessment***
    - A detailed neurological assessment was done at 18 months' age using Amiel-Tison examination by a trained physiotherapist. Any child judged to be abnormal in any respect was re-evaluated by a trained pediatric neurologist.
    - Developmental assessment was done using DASII-2 by trained and certified clinical psychologists. Additionally, social maturity using the Indian adaptation of Vineland social maturity scale (VSMS), behavior using the Child Behavioral Checklist (CBCL-LDS 11/2) and autism screening using M-CHAT were also done at 18 months of age.
    - Visual assessment was done by an experienced pediatric ophthalmologist, who examined the infant for vision, squint, cataract and optic atrophy. Visual acuity was measured using Cardiff visual acuity cards between 9 to 18 months of age by a trained optometrist.
    - For assessment of hearing, a two-step Automated Auditory Brainstem Evoked Response (AABR) test was done using MB11 BERAPhone prior to discharge; hearing and acquisition of receptive and expressive language milestones were assessed at 18 months of age.
    - All outcome assessors were blinded to the gestational age and perinatal history of the child.
    - For the secondary outcome of growth, weight, length and head circumference were recorded as per the WHO-MGRS study procedures and the respective z-scores were derived: the z scores were based on the Fenton growth charts for preterm infants at birth, discharge and 40 weeks of postmenstrual age, and subsequently on WHO- MGRS growth standards.
    - Undernutrition was defined as weight-for-age z score <2 SD, stunting length-for-age z score <2 SD and wasting as weight-for-length <2SD as per WHO-MGRS growth standards.
- ***Quality assurance***
    - Developmental assessments were done by trained and certified clinical psychologists with inter-observer variability of <5%.
    - The assessments were also cross-verified by a senior, experienced child psychologist at regular intervals.
    - Anthropometry was done by a trained team, with inter-observer variability for weight being less than ±5g, length ±1cm and head circumference ±0.5cm. Anthropometry was regularly cross-verified by a senior pediatrician, who served as the gold standard.
    - All enrolment forms were verified by senior pediatrician for very preterm group, and 10% of randomly selected forms for the term AGA group.
    - All infants with abnormal neurological examination were re-evaluated by a pediatric neurologist.
- ***Data management and statistical analysis***
    - We enrolled a convenient sample of 250 each of very preterm and term AGA infants in view of resource constraints.
    - Data were recorded in pre-designed, pre-tested proforma and entered in a database created in Visual Basic with inbuilt range and logistic checks. Double data entry was done.

      Analysis was done using Stata 11 (STATA Corp USA, Tx). Continuous, normally distributed data were analyzed using Student t-test, categorical data using chi-square test, and non-normally

distributed data using Wilcoxon ranksum test for continuous and McNemar test for categorical data. Multivariate logistic regression analysis was done with neurodevelopmental disability as dependent variable and various morbidities at birth as predictors.

Key results

- A total of 820 infants were enrolled in the study: 212 very preterm, 254 late preterm, 87 term SGA, 17 term asphyxiated, and 250 term healthy infants.
- Of these, a total of 776 infants (follow-up rate of 94.6%) were assessed at 18 months of age for the final outcome.
- ***Baseline characteristics***
  - Baseline characteristics of the study infants in different categories are given in Table 11. Among the demographic variables, there was a significant difference in maternal education and family income between the groups, with higher proportion of infants in the very preterm and term SGA cohorts having lower maternal education, and lower family income.
  - There was a significant difference in birth weight, gestation and in-hospital morbidities between the groups, as expected. A higher number of infants in very preterm and term SGA groups were males, and most in-hospital morbidities were present in very preterm infants.

Table 11: Baseline characteristics

Variables	Very preterm (n=212)	Late preterm (n=254)	Term SGA (n=87)	Term asphyxia (n=17)	Term AGA (n=250)
Mother age (in years)	28.1±5.1	27.6±4.0	27.8±4.0	28.7±4.7	27.3±4.0
Father age (in years)	31.2±5.6	30.8±4.5	31±4.4	31.2±4.4	30.4±4.2
Maternal education					
Professional/Graduate/PG	95 (45.2)	127 (50.0)	40 (46.0)	10 (58.8)	149 (59.8)
Inter/Post-Sec/diploma	43 (20.5)	48 (18.9)	13 (14.9)	3 (17.6)	55 (22.1)
10^{th} /8^{th} /Primary/Illiterate	72 (34.3)	79 (31.1)	34 (39.1)	4 (23.5)	45 (18.1)
Family income (INR/month)	n=210	n=252	n=86	N=16	n=249
> 19575	107 (50.9)	127 (50.4)	39 (45.3)	12(75.0)	163 (65.4)
9788-19574	45 (21.4)	77 (30.5)	22 (25.6)	4(25.0)	58 (23.3)
7323-9787	14 (6.7)	10 (4.0)	8 (9.3)	0	15 (6.0)
<7323	44 (20.9)	38 (15.1)	17 (19.8)	0	13 (5.2)
Birth weight (g)	1294±357	2259±459	2023±200	2824±362	2825±298
Birth weight categories					
<1000	51(24.1)	0	0	0	0
1000 to 1499 g	105 (49.5)	17 (6.7)	1 (1.1)	0	0
1500 to 1999 g	50 (23.6)	49 (19.3)	40 (46.0)	0	0
2000 to 2499 g	6 (2.8)	108 (42.5)	46 (52.9)	2 (11.7)	42 (16.8)
≥2500	0	80 (31.5)	0	15 (88.2)	208 (83.2)
Gestation (weeks)	30.4±2.0	35.2±1.0	38.0±1.0	38.3±1.1	38.1±1.0
Male gender	122 (57.5)	126 (49.6)	33 (37.9)	10 (58.8)	118 (47.2)
Multiple births (twin/triplet)	44 (20.8)	65 (25.6)	7 (8.0)	1 (5.9)	11 (4.4)
Intrauterine growth category*					
AGA	143 (67.4)	183 (72.0)	0	17(100)	250 (100)
SGA	61 (28.8)	29 (11.4)	86 (100)	0	0
LGA	8 (3.8)	42 (16.5)	0	0	0
Respiratory distress syndrome	70 (33.0)	3 (1.2)	0	0	0
Chronic lung disease	29 (13.7)	1 (0.4)	0	0	0
Culture positive sepsis	18 (8.5)	1 (0.4)	0	0	0
Shock requiring vasopressors	17 (8.0)	0	0	2 (11.7)	0
HIE stage 2 or more	2 (0.9)	0	1 (1.1)	7 (41.2)	1 (04)

Intraventricular hemorrhage	25 (11.8)	1(0.4)	0	0	1 (0.4)
NEC stage 1 and more	14 (6.6)	0	1 (1.1)	0	1 (0.4)
Symptomatic hypoglycemia	5 (2.4)	3 (1.2)	3 (3.4)	0	0
Symptomatic polycythemia	12 (5.7)	5 (2.0)	1 (1.1)	0	0
NICU stay (days)	20 (10-38.8)	1(0-3)	0 (0-2)	2(1-4)	0
Hospital stay (days)	8 (5-12)	5(3-7)	5 (4-6)	5 (2-6)	4(3-4)

**Using AIIMS intrauterine growth charts*
Abbreviations: NICU, neonatal Intensive care unit; AGA, appropriate for gestational age; SGA, small for gestational age; LGA, large for gestational age; NEC, necrotizing enterocolitis; HIE, hypoxic ischemic encephalopathy
Data expressed as number (percentage) or median (range) or mean±SD; p-values using ANOVA

- ***Primary outcome: Neurodevelopmental impairment at 18 months of age***
  - Primary outcome was ascertained at a mean age of 18.6±1.7 months.
  - Major disability (cerebral palsy or developmental delay or hearing or vision impairment or epilepsy) was present in 11.3% of very preterm and 5.4% of late preterm infants at 18 months of age (Table 12). Among term infants, the rates of disability were lower: 6.0% of term asphyxiated, 3.7% of term SGA and 2.9% of term AGA infants had disability at 18 months of age (Table 12).

Table 12. Incidence of major disability at 18 months of corrected age

Major disability	Very preterm (n=195)	Late preterm (n=239)	Term SGA (n=80)	Term asphyxia (n=17)	Term AGA (n=239)	P
Cerebral palsy (CP)						
None	190 (97.4)	233 (97.5)	78 (97.5)	17 (100)	236 (98.7)	
Spastic diplegic CP	3 (1.5)	2 (0.8)	1 (1.2)	0	1 (0.4)	0.91
Hemiplegic CP	1 (0.5)	1 (0.4)	0	0	0	
Non-classifiable CP	1 (0.5)	3 (1.3)	1 (1.2)	0	2 (0.8)	
Developmental delay*	**n=180***	**n=235***	**n=75***	**n=16***	**n=231***	
None	162 (90.0)	227 (96.6)	72 (96.0)	15 (93.7)	228 (98.7)	
Global (Motor and Mental DQ<70)	5 (2.8)	6 (2.5)	1 (1.3)	0	3 (1.3)	<0.01
Isolated motor (Motor DQ<70)	12 (6.6)	2 (0.8)	2 (2.7)	0	0 (0)	
Isolated mental (Mental DQ<70)	1 (0.6)	0 ()	0	1 (6.2)	0	
Hearing impairment requiring hearing aid	0	0	0	0	1 (0.4)	1.0
Blindness	0	0	0	0	0	-
Epilepsy (>1 one episode of unprovoked seizure)	0	0	0	0	1 (0.4)	1.0
Any disability	22 (11.3)	13 (5.4)	3 (3.8)	1 (5.9)	4 (1.7)	0.001
DQ unsatisfactory	15 (7.7%)	4 (1.7)	5 (6.3)	1 (5.9)	8 (3.3)	-

**DQ could not be performed satisfactorily in 13 (6.7%) of very preterm, 4 (1.7%) of late preterm, 5 (6.3%) of term SGA, 1 (5.9%) of asphyxiated and 8 (3.3%) of term AGA infants due to various reasons like sickness, noncooperation or behavioral problems- the data for these infants has been excluded from present analysis.*

  - The mean motor and mental developmental quotients between the groups were within normal range. There was a statistically significant difference mean motor and mental developmental quotients between the groups, but it was not clinically significant/relevant (Table 13).
  - A significantly higher proportion of very preterm infants had motor developmental delay (MoDQ <70; 10.0%) compared to other groups (late preterm 3.4%, term SGA 4.0% and term AGA 3.0%, and none in the asphyxia group).
  - However, developmental delay in the mental domain was highest in the infants with asphyxia (6.2%), followed by late preterm (2.5%) and very preterm infants (2.2%). Only 1.3% infants in term SGA and less than 1% of term AGA infants had developmental delay in the mental domain (Table 13).

Table 13. Motor and mental developmental quotients at 18 months of age by study cohorts

	Very preterm (n=195)	Late preterm (n=239)	Term SGA (n=80)	Asphyxia (n=17)	Term AGA (n=239)	P
Motor DQ (50th centile)	64.1±16.1	97.8±12.0	94.7±12.4	99.4±9.2	97.4±11.2	0.02
Motor DQ (50th centile) <70 70-84 ≥85	 18 (10.0) 23 (12.8) 139 (77.2)	 8 (3.4) 22 (9.4) 205 (87.2)	 3 (4.0) 14 (18.7) 58 (77.3)	 0 1 (6.2) 15 (93.7)	 3 (1.3) 23 (10.0) 205 (88.7)	 0.001
MeDQ (50th centile)	98.3±13.3	101.9±13.3	102.0±12.3	100.0±11.5	101.6±11.5	0.03
MeDQ (50th centile) <70 70-84 ≥85	 4 (2.2) 16 (8.9) 161 (88.9)	 6 (2.5) 10 (4.3) 219 (93.2)	 1 (1.3) 3 (4.0) 71 (93.7)	 1 (6.2) 0 (0) 15 (93.7)	 2(0.9) 8(3.5) 221(95.7)	 0.16

Data expressed as number (percentage), median (range) or mean±SD

(DQ, developmental quotient)

- ***Secondary outcomes: Growth from birth until 18 months of age***
  - Very preterm infants showed a decline in weight, length and head circumference z-scores from birth until 3 months of corrected age followed by some improvement until 18 months of age. However, these infants did not acquire the birth growth centiles even until 18 months of age (Figure 9).
  - Term SGA infants showed a fall in weight, length and head circumference z-scores from birth until discharge, followed by an improvement until 9 months of age; thereafter the values remained stationary until 12 months, followed by some improvement in head circumference, but a marginal fall in the z-scores for weight and length (Figure 10).
  - As for late preterm infants, there was a small decline in mean weight, length and head circumference z-scores from birth until 18 months of age (Table 14).
  - Among term AGA infants, the mean z-scores for weight and length improved from birth until 18 months of age while that for head circumference for age remained similar (-0.8 at each time point).

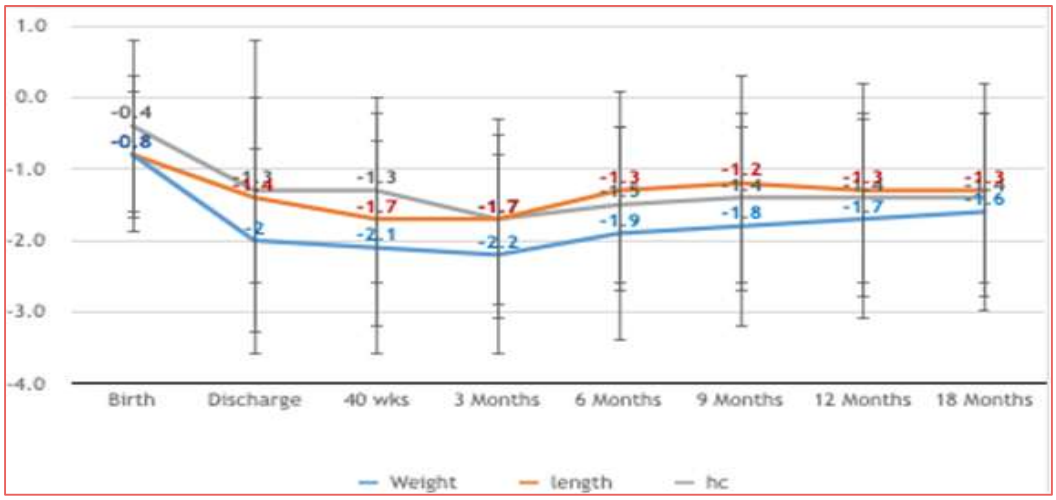

Figure 9: Anthropometry z-scores over time for 'Very preterm' infants

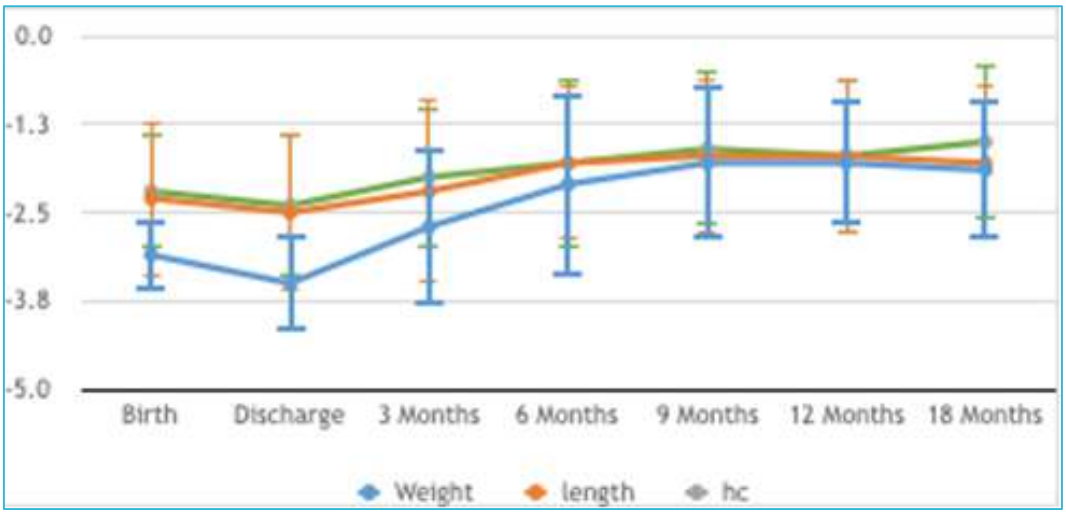

Figure 10: Anthropometry z-scores over time for 'Term SGA' infants

Table 14. Weight, length and head circumference z-scores of study infants

Z-scores*	Very preterm	Late preterm	Term SGA (n=58)	Asphyxia (n=17)	Term AGA (n=250)	P
Weight for age						
Birth	-0.8±0.9	-0.8±0.10	-3.1±0.5	-1.1±0.81	-1.0±0.7	<0.001
Discharge	-2.0±1.3	-1.5±1.3	-3.5±0.7	-1.6±0.84	-0.5±0.8	<0.001
18 Months	-1.6±1.4	-1.0±1.1	-1.9±1.0	-0.3±1.8	-0.8±1.1	<0.001
Length for age						
Birth	-0.8±1.1	-0.7±1.5	-2.3±1.1	-1.0±1.0	-1.0±1.6	<0.001
Discharge	-1.4±2.2	-0.8±1.6	-2.5±1.1	-1.3±0.9	-1.0±1.6	<0.001
18 Months	-1.3±1.5	-0.9±1.3	-1.8±1.1	-0.5±1.4	0.8±1.2	<0.001
Head circumference	N=239					
Birth	-0.4±1.2	-0.6±0.9	-2.2±0.8	-0.7±1.1	-0.8±1.2	<0.001
Discharge	-1.3±1.3	-0.7±1.1	-2.4±1.0	-1.0±1.1	-0.9±1.3	<0.001
18 Months	-1.4±1.2	-1.0±1.1	-1.5±1.1	-0.6±0.9	-0.8±1.0	<0.001

- There was little catch up growth (defined as change in growth parameter SDS scores of >0.67 over time) in weight, length and head circumference during the study period among the very and late preterm infant cohorts (catch up in weight: 11.8% and 23.0%; catch up in length 19.9% and 23.0%, and catch up in head circumference 7.2% and 13.4% in very preterm and late preterm cohorts respectively; Table 14).
- Despite the fact, a higher proportion of infants in the term SGA cohort had undernutrition (weight for age z-score less than -2SDS) and stunting (length for age z score less than -2 SDS) at 18 months of age compared to the very preterm and late preterm infants (Table 14).

- ***Neuroimaging data***
  - The data on a total of 101 MRIs and 102 cranial USGs on the very preterm infants (both MRI and simultaneous cranial USGs in n=87) was collected as part of the study. The format for analysis and reporting of the data has been finalized in consultation with the radio diagnosis team and have been reported by two independent observers, separately for cranial USGs and MRIs. The data is being finalized.

Key conclusions

- The overall rates of disability were low compared to those reported previously from similar and even more developed settings. However, the disability rates among infants <28 weeks and <1000 g remain high.
- In a tertiary care setting with adequate quality of care through the postnatal period (which includes in-hospital and post-discharge early intervention for infants in need), it is feasible to achieve low rates of disability even among high risk preterm infants.
- However, postnatal growth of preterm and term SGA infants continues to remain an important concern, with few of the preterm infants showing catch up growth. Though the catch up growth was better in the term SGA infants, a significant proportion of preterm and term SGA infants remain undernourished and stunted at 18 months of age

ADDITIONAL PROJECTS ON FOLLOW-UP OF HIGH-RISK NEONATES

1. Age at attainment of developmental milestones in preterm infants

Rationale: Controversy still remains whether the attainment of developmental milestones are delayed even after correction for the period of prematurity in preterm infants born before 33 weeks' gestation. Not many studies from India have looked at this aspect in a systematic manner.

Research question

Are the developmental milestones of very preterm infants (28 to 32 weeks' gestation) without any major morbidities delayed when compared to reference norms of term healthy neonates?

Objective: To compare the age at achievement of different developmental milestones in the first 6 months of corrected age in very preterm (28-32 weeks) infants with the reference norms for Indian infants (Developmental Assessment Scale for Indian Infants, DASII).

Methods26

- In this prospective observational study, a convenient sample of 35 healthy preterm infants (gestation 28-32 weeks) was prospectively followed from birth until 6 months of corrected age (CA).
- Developmental assessment was done serially, beginning at 40 weeks post menstrual age, every 15 days in first 3 months and monthly till 6 months CA. The assessment was blinded, using items in DASII scale (validated Indian adaptation of BSID-II), classified into 4 main domains of development.
- We selected a total of 66 key milestones from the standard DASII proforma for developmental assessment under the present study.
- The age at attainment of these milestones was calculated and expressed in months. Each 0.1 represents 3 days. To compare the statistical significance, we calculated the mean age at attainment of the milestones by our study infants, and compared it with the corresponding 50th centile of that milestone on DASII scale, which was taken as mean.

Results

- A total of 39 neonates were enrolled in the study, of whom 35 underwent developmental assessment as scheduled at every 15-day interval in the first 3 months of corrected age and monthly till 6 months corrected age.
- The mean gestational age of the enrolled infants was 30.9 weeks and mean birth weight was 1345 g, with 20% being small for gestational age (SGA).
- Of the total 66 key milestones selected from the standard DASII proforma for developmental assessment, 32 milestones (48.5%) were attained by all neonates, 28 milestones (42.4%) were achieved by some infants, while 6 of the 66 milestones (9.1%) were not achieved in any of the study infants during the study period.
- Of the 32 milestones attained by all infants during the study period, the mean age at attainment of 23 milestones (71.9%) was delayed compared to the 50th centile of the DASII norms for term healthy infants. Five (15.6%) out of 32 milestones were attained earlier compared to the 50th

centile of DASII and 4 milestones were attained at an age comparable with the 50th centile of DASII norms (12.5%).

Conclusion: Even after correcting for prematurity, "healthy" preterm infants failed to attain almost 72% of test items in different domains as per the reference norms for term healthy infants, and required early intervention. Further studies with larger sample size and longer term follow up are required to obtain normative values for these children.

2. Diagnostic performance of the Bayley Infant Neurodevelopmental Screener (BINS) as a neurodevelopmental screening tool for high-risk infants in India

Rationale: There is a clinical need for a robust neurodevelopmental screening tool for high risk infants, easy enough to be used in busy clinical practice. There is no data available from India on the diagnostic performance of Bayley Infant Neurodevelopmental Screener (BINS). We tested the diagnostic utility of BINS by comparing it against the Developmental Assessment Scheme for Indian Infants (DASII), Indian adaptation of Bayley-II, as the "gold standard".

Research question

What is the sensitivity of BINS as compared to a reference test DASII for diagnosing developmental delay among high-risk infants (discharged from a tertiary care unit) between 3-24 months corrected age?

Objectives

Primary: To determine the sensitivity of BINS, in comparison to DASII as 'Reference test' in the detection of developmental delay in high-risk infants discharged from a tertiary care unit, between 3-24 months corrected age.

Secondary: To determine the specificity and likelihood ratios of BINS, in comparison to DASII as 'Reference test', in the detection of developmental delay in high-risk infants discharged from a tertiary care unit, between 3-24 months corrected age.

Methods

The present cross-sectional study was conducted in the high risk follow up clinic of the tertiary care neonatal unit at the All India Institute of Medical Sciences, Delhi, India.

Results

A total of 91 infants were enrolled in the study between March 26th, 2014, and August 31st, 2015. The baseline characteristics of the study infants are provided in Table 1. Mean gestation of the infants was 34 weeks and birth weight 1938 g.

Primary outcome: Sensitivity of the test was 90.9% based on composite DQ, 89.6% based on motor DQ alone, and 94.9% based on mental DQ alone.

Conclusion: Bayley Infant Neurodevelopmental Screener had a good sensitivity but poor specificity in screening for the risk of developmental delay among high risk infants. Since the present study had small numbers, further studies with larger sample size are required to decide upon the routine use of BINS for screening high risk infants with BINS in busy clinical settings.

3. Diagnostic performance of distortion product and transient evoked oto-acoustic emissions for hearing screening

Rationale: Otoacoustic emissions (OAEs) are a feasible alternative for hearing screening of high risk neonates in view of it being a quick, portable bedside test which can be done with little training. However, there is paucity of robust data on their diagnostic utility among high risk neonates.

Objectives

Primary: To determine the diagnostic performance of distortion product otoacoustic emission (DPOAE) compared to two step automated auditory brainstem response (AABR) as gold standard to determine hearing loss at 40 dB in high risk neonates

Secondary: To determine the diagnostic performance of transient evoked otoacoustic emission (TEOAE) compared to two step automated auditory brainstem response (AABR) as gold standard to determine hearing loss at 40 dB in high risk neonates

Methods

- The study was conducted in the Department of Pediatrics at AIIMS, Delhi from Oct 2013 to April 2015. Ethical clearance was obtained from the institutional ethics committee. All high-risk infants underwent simultaneous hearing screening using TEAOE, DPOAE and AABR prior to discharge.
- OAEs (both TEOAE and DPOAE) were tested using portable GSI AUDIOscreener. AABR was done using BERAphone MB11® on the same day as OAEs; The personnel doing AABR were blinded to the OAE results.
- Those infants who had a "refer" result either unilaterally or bilaterally on the first screen were re-subjected to a second AABR screen within 48 hours of the first screen. The second screen was performed on both the ears even if the initial screen had only unilateral refer result, as per the JCIH guidelines. Infants who were "refer" on the second screen also (either unilateral or bilateral), were considered to have hearing impairment for the purpose of the present study, and were also referred for a diagnostic BERA in the Department of ENT.

Results

- A total of 196 infants qualified, of whom 103 could be enrolled in the study; of these, one had unilateral anotia, so a total of 205 ears were tested by all the three modalities- DPOAE, TEOAE and AABR.
- Of the 143 ears tested with both DPOAE and a two-step AABR, it was a total of 3 ears were 'refer' on both (true positive) and 118 ears were 'pass' on both (true negative). Two ears were 'refer' on DPOAE but passed on AABR (false positive 1.6 %) and 20 ears passed on DPOAE which were refer on AABR (false negative 86.9 %).

  The diagnostic parameters of DPOAE accordingly, are as follows:
  - Sensitivity = 3/23= 13% (95% CI 2.8 to 33.6%)
  - Specificity = 118/120= 98.3% (95% CI 94.1 to 99.8%)
  - Positive predictive value= 3/5*100=60%
  - Negative predictive value= 118/138*100=85.5%
  - Likelihood ratio positive= sensitivity/1-specificity= 7.64
  - Likelihood ratio negative= 1-sensitivity/specificity= 0.88
- Of the 143 ears tested with both TEOAE and a two-step AABR, it was a total of 12 ears were refer on both (true positive) and 110 ears were pass on both (true negative). Ten ears were 'refer' on TEOAE but passed on AABR (false positive 8.3 %) and 11 ears passed on TEOAE which were refer on AABR (false negative 47.8 %)

  The diagnostic parameters of TEOAE accordingly, are as follows:
  - Sensitivity = 12/23= 52.2% (95% CI= 30.6 to 73.2%)
  - Specificity = 110/120 =91.6% (95% CI 85.2 – 95.9%)
  - Positive predictive value=12/22*100= 54 %
  - Negative predictive value= 110/121*100 = 90.0 %
  - Likelihood ratio positive= 30.5
  - Likelihood ratio negative= 0.48
- The agreement between DPOAE and TEAOE was also low - kappa coefficient between the two of 0.33 for right ear and 0.27 for the left ear.

Conclusion: Our study shows that OAEs have low sensitivity and high specificity for hearing screening among high risk neonates, when compared to a two-step AABR, with TEOAE performing better than DPOAE. The present results primarily serve as pilot data to inform further studies to test the diagnostic utility of OAEs/protocols using OAEs among high risk neonates.

4. Feasibility of implementing universal newborn hearing screening at a tertiary care centre in India

Background: We conducted a feasibility study on implementing universal newborn hearing screening at AIIMS, New Delhi. The paper has been published and is attached in the Annexure (*Gupta S, Sah S, Som T, Saksena M, Yadav CP, Sankar MJ, Thakar A, Agarwal R, Deorari AK, Paul VK. Challenges of Implementing Universal Newborn Hearing Screening at a Tertiary Care Centre from India. Indian J Pediatr 2015 Aug;82(8):688-93*). The abstract is presented below:

Objectives**:** To report experience of implementing universal newborn hearing screening (UNHS) in a tertiary care neonatal unit, identify risk factors associated with failed two-step automated acoustic brainstem response (AABR) screen and evaluate cost of AABR.

Methods: This was a prospective study of UNHS outcomes of all live births with two step AABR using BERAphone MB11®. Outcome measures were screening coverage, refer, pass and lost to follow up rates and cost of AABR using micro-costing method. To identify risk factors for failed screening, authors performed multivariate logistic regression with failed two-step AABR screen as dependent variable and baseline risk factors significant on univariate analysis as predictors.

Results: Screening coverage was moderate (84 %), with 2265 of total 2700 eligible infants screened with initial AABR (mean gestation 37.2 ± 2.3 wk; birth weight 2694 ± 588 g; 305 received nursery care). A total of 273 of 2265 infants were "refer" on first screen. Second screen was done on 233, of which 58 were "refer". Of these, 35 underwent conventional ABR, of which 5 were diagnosed to have hearing impairment. Only 2 could get hearing aid. Overall, a total of 2197 (81.4 %) infants passed, 496 (18.4 %; excluding 2 deaths) were lost to follow up at various stages, and 5 (0.2 %) were diagnosed with hearing impairment, all of whom were high risk. Average cost of AABR was INR 276 per test. No factor emerged as significant on multivariate analysis.

Conclusions: UNHS is feasible to implement, but significant lost to follow up and non-linkage with appropriate rehabilitation services limit its utility. Cost effectiveness of UNHS compared to high risk based screening needs to be determined

THEME 4: CAPACITY BUILDING

PROJECT 5: FACILITATING DEVELOPMENT OF NEW MULTI-SITE STUDIES ON NEWBORN HEALTH BY RESEARCHERS OF OTHER INSTITUTIONS IN THE COUNTRY

Background

The ICMR supported the establishment of the National Neonatal-Perinatal Database (NNPD) Network of 18 leading neonatal care institutions providing information on one of the largest cohort of 150,000 neonates in India. We proposed to take this process forward as a catalyst. The Centre would facilitate development of multi-site studies. Other institutions would lead development of these studies – the Centre would only serve as a coordinating point.

Methods

- We organized two protocol development meetings of reputed researchers in Neonatology from different institutions of the country, along with methodology and subject experts from India and other countries and ICMR scientists.
- About 20 experts from different institutions of India attended the meetings.
- The following multi-site protocols were discussed in this meeting:
    - Efficacy and safety of indigenous goat lung surfactant extract for treatment of RDS in preterm babies (Dr VK Paul, AIIMS, New Delhi)
    - Developing postnatal growth standards for Indian very low birth weight infants (Dr Swarna Rekha Bhat, St John's Hospital, Bangalore)
    - Establishing a national neonatal network for quality improvement (Dr Deepak Chawla, GMCH, Chandigarh)

Outputs

Indigenous surfactant trial

- The multi-site protocol on "Efficacy and safety of indigenous goat lung surfactant extract for treatment of RDS in preterm babies" was submitted for funding under 'Affordable healthcare initiatives' scheme of the Wellcome Trust (WT), UK.
- WT has approved the proposal and has sanctioned a funding of approx. 11 crore INR.
- We plan to do a multi-centric, double-blinded, non-inferiority randomized controlled trial (RCT) in preterm infants with respiratory distress syndrome (RDS) (n~900) to test the hypothesis that goat lung surfactant (GLSE) is non-inferior to the standard preparation of Survanta (Survanta®, Abbott, USA) for treating RDS in preterm infants.
- The objectives of the trial are:
    - *Primary objective:* To compare the incidence of BPD free survival at 36 weeks of postmenstrual age (PMA) in preterm neonates (<32 weeks) with RDS randomized to receive intratracheal administration (100 mg/kg) of either GLSE (Cadisurf®) or beractant (Survanta®).
    - *Secondary objectives:* (1) To compare the area under curve (AUC) for oxygen requirement (FiO2) in first 48 h of surfactant administration in a subset of 250 infants; (2) To compare the incidence of air leaks, pulmonary hemorrhage, neonatal mortality, healthcare associated infections, cerebral white matter abnormalities and retinopathy of prematurity (all neonates)
- The trial will be conducted as per guidelines provided by DCGI and other regulatory agencies and with an oversight by an independent DSMB. Additionally, an International Advisory Board (IAB) led by Prof MK Bhan would supervise the study planning, implementation and scientific outcome of the study.
- The study will be conducted in 12 sites (including AIIMS, New Delhi) all over India in collaboration with Clinical Development Service Agency (CDSA), an autonomous institute under the Department of Biotechnology, Government of India.

- We have obtained the necessary clearances from the Ethics Committees of the concerned institutions, Central Drugs Standard Control Organization (CDSCO), and Health Ministry's Screening Committee, ICMR.
- Enrolment has been started from 1st July 2016.
- The trial would help introduction of GLSE to clinical practice filling an important availability gap of a life-saving drug salvaging many newborn lives in India.

Project 6: Enhancing research methodology skills of faculty at teaching institutions

Aim

Our aim was to improve the quality of medical research in the field of neonatal and perinatal medicine so as to generate evidence of high standards addressing the key issues pertaining to these fields.

Objectives

To improve the quality neonatal-perinatal research through capacity strengthening in the teaching institutions of the country by:

- Enhancing skills of the faculty of teaching institutions in research methodology and clinical epidemiology
- Enhancing skills of the faculty of teaching institutions in mentoring postgraduate theses in pediatrics/ neonatology/nursing/OBGYN
- Providing technical support and guidance through a research facilitation node

Methods

The following activities were proposed:

- Research methodology workshops: We planned to undertake research methodology workshops to help researchers in generating relevant research questions, identify priority areas in neonatal and perinatal medicine, and enhance their capacity to undertake high quality research at respective institutions. The workshops would particularly focus on postgraduate theses-linked research. The format of workshop would be hands-on training. Exercise would include protocol development, discussion and analysis of the projects.
- Developing a system by which we would provide facilitation services by offering to review protocols and providing feedback to the researchers on design and analysis in the teaching institutions thorough emails and tele-consultation.
- Developing a toolkit for research. We would design and maintain a website for dissemination of this toolkit to a wider audience.

Outputs

- Two research methodology workshops were conducted – one each in Delhi and Bangalore – to help young faculty working in medical colleges from different parts of the country.
- Both were attended by 35-40 young faculty from all over India.
- The participants expressed immense satisfaction on the objectives and processes of the workshop.
- In order to evaluate the effectiveness of the workshops, we conducted a survey almost a year after the completion of the workshops. A series of multiple-choice questions were framed with semi-quantitative responses.
- The survey was mailed to all the participants along with the objective of the survey and a request to fill in the same. In case of non-response, reminder mails and telephonic calls were done.
- The overall response rate was 45/67 (67%). The findings of the survey are enumerated below (Table 15):

Table 15: Survey results of the research methodology workshops

		Bangalore & workshop, n=(27+18)=45	
1	Increase in KNOWLEDGE on research methods	• Very highly (80-100%), n=9 (20%) • Highly (by 60-80%), n=31 (69%) • Average (40-60%), n=5 (11%)	
2	Increase in SKILLS of research methods	• Very highly (80-100%), n=5 (11.1%) • Highly (by 60-80%), n=28 (62.2%) • Average (40-60%), n=12 (26.7%)	
3	How many of the protocols (thesis/others) received funding	***Before attending workshop*** • Zero, n=38 (84.4%) • One, n=5(11.1%) • Two, n=1 (2.2%) • Three, n=1(2.2%)	***After attending workshop*** • Zero, n=32 (71.1%) • One, n=6 (13.3%) • Two, n=5 (11.1%) • Three, n=2 (4.4%)
4	In which of the following areas of research methodology has your knowledge/skills improved significantly following the workshop?		
	Better identification of gaps in knowledge and, research needs	• Improved very significantly, n=8 (17.8%) • Improved significantly, n=31 (68.9%) • Improved marginally, n=5 (11.1%) • Did not improve, n=1 (2.2%) • Further confused me. N=0	
	Review of literature	• Improved very significantly, n=7 (15.6%) • Improved significantly, n=27 (60.0%) • Improved marginally, n=11 (24.4%) • Did not improve, n=0 • Further confused me, n=0	
	PubMed search	• Improved very significantly, n=11 (24.4%) • Improved significantly, n=19 (42.2%) • Improved marginally, n=15 (33.3%) • Did not improve, n=0 • Further confused me, n=0	
	Writing protocol, e.g., detailed methodology	• Improved very significantly, n=13 (28.9%) • Improved significantly, n=27 (60.0%) • Improved marginally, n=4 (8.9%) • Did not improve, n=1 (2.2%) • Further confused me, n=0	
	Sample size calculation	• Improved very significantly, n=5 (11.1%) • Improved significantly, n=18 (40.0%) • Improved marginally, n=15 (33.3%) • Did not improve, n=7 (15.6%) • Further confused me, n=0	
	Involvement of statistician at protocol development stage	• Improved very significantly, n=9 (20.0%) • Improved significantly, n=22 (48.9%) • Improved marginally, n=12 (26.7%) • Did not improve, n=2 (4.4%) • Further confused me, n=0	
	Ethics clearance	• Improved very significantly, n=4(8.9%) • Improved significantly, n=27 (60.0%) • Improved marginally, n=12 (26.7%) • Did not improve, n=1 (2.2%) • Further confused me, n=1 (2.2%)	

	Using proper database, such as MS Access	• Improved very significantly, n=4 (8.9%) • Improved significantly, n=18 (40.0%) • Improved marginally, n=15 (33.3%) • Did not improve, n=8 (17.8%) • Further confused me, n=0
	Using statistical software such as STATA, Epi info etc.	• Improved very significantly, n=4 (8.9%) • Improved significantly, n=11 (24.4%) • Improved marginally, n=20 (44.4%) • Did not improve, n=10 (22.2%) • Further confused me, n=0
	Better writing	• Improved very significantly, n=10 (22.2%) • Improved significantly, n=20 (44.4%) • Improved marginally, n=14 (31.1%) • Did not improve, n=1 (2.2%) • Further confused me, n=0
5	Conducted any further workshop to disseminated knowledge/skills gained	• None, n=26 (57.8%) • One, n=9 (20.0%) • Two, n=7 (15.6%) • Three, n=1 (2.2%) • Four, n=2 (4.4%)
6	Do you think there is a need to conduct such workshops in future?	• Yes, twice a year, n=15 (33.3%) • Yes, once a year, n=15 (33.3%) • Yes, frequency not specified, n=8 (17.8%) • Other s, n=7 (15.6%)
7	Should these workshops be on payment or sponsored by organizations like ICMR	• On payment, n=12 (26.7%) • Sponsored, n=33 (73.3%)
8	Research facilitation cell	• Is a must, n=30 (66.7%) • Might possible help, n=11 (24.4%) • Not sure if it would help, n=3 (6.7%) • No need for any further facilitation, n=1 (2.2%)

**Data presented as number (percentage)*

THEME 5: TECHNICAL ASSISTANCE TO ICMR

Project 7: Developing bio-ethics guidelines on child health research in India

Background

- The Indian Council of Medical research (ICMR) brought out the 'Policy Statement on Ethical Considerations involved in Research on Human Subjects' in 1980 and revised these guidelines in 2000 as the 'Ethical guidelines for Biomedical Research on Human Subjects'. The third version was developed in 2006.
- The ICMR guidelines do have a small section pertaining to research in children; however, a need was felt to develop more comprehensive detailed guidelines, which pertain to the specifics of ethics in biomedical research in neonates and children.

Objectives

a. To create bioethics guidelines for conduct of research involving newborn and child participation including drug trials.

b. To establish neonate and child specific Standard Operating Procedures (SOP) to measure and provide accountability regarding the quality and effectiveness of ethical review in our country at par with international quality.

Methods

- As a first step, the existing national and international guidelines for biomedical research in children were reviewed. Separate guidelines available for pediatric biomedical research in other countries include the Institute of Medicine (IOM) guidelines in the USA, the Medical Research Council (MRC) guidelines in the United Kingdom, and the European Union (EU) guidelines. These guidelines have been reviewed for a better understanding of the ethical principles of biomedical research in children.
- Members of the CAR team including Dr Suvasini Sharma, Assistant Professor of Pediatrics at Kalawati Saran Children Hospital, New Delhi and Dr Naveen Sankhyan, Assistant Professor of Pediatrics at PGIMER, Chandigarh, prepared the draft guidelines in March 2011
- The report was discussed in an Expert Group Meeting in April 2011; the comments were addressed and the first draft report was sent for feedback.
- The expert group was further expanded to include representation from judiciary and Prof Richard Cash (Harvard School of Public Health).

Outputs

- The final document "National Ethics Guidelines for Biomedical Research involving Children" has been submitted to ICMR for discussion, feedback, and finalization and dissemination.
- The document covers the ethical and legal issues that researchers need to consider when carrying out biomedical research in neonates and children. The aim has been to set out general principles that can be applied in most situations rather than to cover each and every situation.
- These guidelines need to be used in conjunction with the current national guidelines for ethical conduct of biomedical research drafted by ICMR and Department of Health Research (DHR), Government of India. They are meant for use by researchers, ethical committees and other involved stakeholders.

Project 8: Providing technical assistance to ICMR in neonatal health

The Division of Neonatology at AIIMS has been providing ongoing technical assistance to the ICMR on a host of areas. The faculty members of the Division are on several technical groups of the ICMR, including:

- High powered Committee on Independent Evaluation of ICMR
- Studies on pneumonia etiology
- Selection committees for the ICMR scientific staff and Director of ICMR institutions
- SAC of the NAREH, Bhopal
- SAC of DMRC, Jodhpur
- Steering Committee of the Indo – US Joint Working Group on maternal and child health research.
- Studies on etiology of neonatal infections
- Implementation research in newborn health
- PRC on prematurity
- PRC on newborn and young infant feeding
- KMC in community settings

CONTRIBUTIONS TO SUBJECT KNOWLEDGE

Theme	Project	What was already known	What this study adds
Neonatal sepsis	*Developing a neonatal sepsis registry*	▪ Sepsis is one of the commonest causes of neonatal deaths globally ▪ Most sepsis-related deaths occur in low- and middle-income countries (LMICs). ▪ There is a paucity of high-quality surveillance networks – a critical element to accurately determine the burden and characterizing the AMR – in LMICs. ▪ Studies landscaping the epidemiology of sepsis and revealing its links to soaring AMR, particularly in the LMICs, are amongst the top research priority areas set by various agencies	▪ Possibly one of the largest studies from the LMIC settings with rigorous methodology, our study confirms the huge burden of sepsis amongst neonates admitted in ICU ▪ Most cases of sepsis occurred within 72 hours of birth. ▪ *Acinetobacter baumannii* emerged as the commonest pathogen, overtaking the 'established' ones like Klebsiella spp. and *E coli* ▪ Most isolates (50% to 80%) of common pathogens were multi-drug resistant (including carbapenems). ▪ The most common gene responsible for carbapenem resistance in Klebsiella and Acinetobacter was NDM-1
	Hand hygiene compliance: Effect of stepwise interventions	▪ Hand hygiene compliance is uniformly poor among health care providers with reported compliance below 50%. ▪ Reported strategies to improve adherence to hand hygiene are not always sustained.	▪ Hand hygiene compliance improves significantly after implementation of a stepwise improvement package. ▪ An incremental effect on compliance observed with standard learning and CCTV monitoring with individual feedback; CCTV monitoring alone didn't improve the compliance rates much
Vitamin D	*Optimal dose of vitamin D in preterm infants*	▪ There is a high prevalence of vitamin D deficiency (VDD) in Indian infants, particularly in LBW and IUGR infants ▪ Recommended RDA of vitamin D in preterm infants ranges from 400 to 1000 IU per day ▪ There is a paucity of evidence regarding the optimal dose of vitamin D supplementation in preterm infants	▪ Daily supplementation with 800 IU of vitamin D reduces the prevalence of vitamin D insufficiency (VDI) at 3 months without having any improvement in bone mineralization. ▪ There is a possibility that this dose may result in vitamin D excess occasionally. ▪ The clinical significance of achieving vitamin D sufficiency needs to be examined in larger trials.
	Optimal dose of vitamin D in term infants	▪ A high prevalence of VDD is observed in term, healthy, exclusively breastfed infants ▪ Current recommendation of 400 IU/day of vitamin D may not be sufficient to ensure optimum serum vitamin D concentration in these neonates. ▪ There is a need to evaluate the efficacy and safety of higher supplemental doses in Indian neonates.	▪ There was a high prevalence of VDD and vitamin D insufficiency (VDI) at 14 weeks of age in term healthy infants supplemented with 400 IU/day of vitamin D3 from birth. ▪ Daily supplementation of 400 IU may possibly be inadequate in these infants. ▪ Only a few infants (<10%) receiving daily supplementation of 800 IU had VDD/VDI at 6 months of age ▪ There was appreciable risk of potential toxicity in some infants; so, this regime cannot be universally recommended in all infants.
LBW nutrition	*Complementary feeding (CF) trial in preterm infants*	▪ Initiation of complementary feeding (CF) serves as watershed in infant nutrient intake with significant potential to affect growth.	▪ Our study shows that early initiation of CF at four month compared to six month of CA:

		▪ There is paucity of evidence on optimal time of initiation of CF in preterm infants.	○ does not improve growth of preterm infants at CA of 12 months. ○ does not result in a difference in neurodevelopment outcomes, body composition, bone mineralization, and any marker for metabolic syndrome like insulin resistance, lipid profile, and blood pressure in infancy ○ increases the risk of hospitalization due to concurrent morbidities, predominantly diarrhea and lower respiratory tract infections. ▪ In both groups, dietary patterns remain poor and body iron stores remain depleted despite iron supplementation till 12 month of CA.
Long-term follow-up of high-risk neonates	*Neurocognitive outcomes of high risk neonates*	▪ There is paucity of long term data on high risk Indian neonates	▪ The major disability was observed in 11.3% of very preterm and 5.4% of late preterm infants. Among term infants, the rates of disability were lower: 6.0% of term asphyxiated, 3.7% of term SGA and 2.9% of term AGA infants had disability at 18 months of age. ▪ There was little catch-up growth (defined as change in growth parameter SDS scores of >0.67 over time) in weight, length and head circumference among very and late preterm infant cohorts ▪ A higher proportion of infants in term SGA cohort had undernutrition and stunting at 18 months of age compared to very preterm and late preterm infants
	Challenges of implementing universal newborn hearing screening *(UNHS)* at a tertiary care centre	▪ There is limited data on the cost-effectiveness, operational challneges in implementation of UNHS in Indian settings	▪ UNHS is feasible to implement, but significant lost to follow up and non-linkage with appropriate rehabilita- tion services limit its utility. Cost effectiveness of UNHS com- pared to high risk based screening needs to be determined.
	Diagnostic utility of oto-acoustic emissions for hearing screening of high risk neonates	▪ paucity of robust data on the diagnostic utility of otoacoustic emissions (OAEs) among high-risk neonates	▪ OAEs have low sensitivity and high specificity for hearing screening among high risk neonates, when compared to a two-step AABR, with TEOAE performing better than DPOAE
	Reducing post-discharge mortality in preterm neonates	▪ We noticed a high post-discharge mortality (70/371; 18.9%) at one of the participating sites in CF trial.	▪ The high post-discharge mortality among preterm VLBW infants may be reduced by timely empowerment of the family coupled with improving access to healthcare through 24x7 telephonic support, which would be of significant importance in resource constrained settings with limited home follow up

TRANSLATIONAL POTENTIAL OF PROJECTS

Projects	Title	Translational potential
1.	Preventing sepsis in high risk neonates	• The multi-pronged strategy demonstrated to improve hand hygiene among healthcare providers in our study can be replicated in other neonatal units including the special care newborn units (SNCUs) established at the district level throughout the country
2.	Developing a neonatal sepsis reference centre/registry, and studying molecular epidemiology of bacterial isolates and their antimicrobial resistance pattern	• High-quality data on the incidence of sepsis, changing profile of organisms, and the alarmingly high rates of antimicrobial resistance in common isolates helps to identify and prioritize the research priorities in neonatal sepsis in India and other low-and middle-income countries (LMICs). • A simplified guide, developed as a part of the study, to facilitate rational use of antibiotics in NICUs can be used in neonatal units across the country as a part of antibiotic stewardship program
3.	Vitamin D status and need for supplementation among LBW infants	• Results of our study would help formulate the guidelines on optimal dose of vitamin D supplementation in preterm LBW neonates
4.	Neurocognitive and physical outcomes of high risk neonates	• Tool-kit for neurodevelopmental follow-up in high-risk infants can be adapted for use in the government initiated RBSK • Findings of our study would help in setting up the research priorities in neurodevelopment of at-risk infants • Evidence-based guidelines to implement early intervention program to improve neurodevelopmental outcomes
5.	Facilitating development of new multi-site studies on newborn health by researchers of other institutions in the country	• The multi-centric phase 3 study involving indigenous surfactant would help reduce the cost of surfactant therapy in India and other LMICs (if found to be effective and safe)
6.	Enhancing research methodology skills of faculty at teaching institutions to stimulate high quality research studies under the postgraduate theses	• Training and mentoring of the young medical faculty in the workshops has helped in better research outputs from the respective units/hospitals • Creating tools including online webinars for research methodology skills will go a long way in educating and training the young faculty of the medical colleges in the country
7.	Developing bio-ethics guidelines on child health research in India	• National bio-ethics guidelines will be a landmark document on the ethics of conducting research in children in the country

APPENDIX

PROJECT 1:

Table 1: Definitions used in the study (adapted from CDC-NHSN)

Diagnosis	Working definitions	Notes/remarks
Culture-positive sepsis (Laboratory-confirmed bloodstream infection)	Blood culture positive AND must meet ANY ONE of the following criteria: **1.** True pathogen detected: Baby has a recognized pathogen cultured from 1 or more blood cultures and organism cultured is not related to an infection at another site AND Physician institutes appropriate treatment for septicemia **2.** CoNS*: CoNS is cultured from 1 or more blood samples drawn on separate occasions (see "Notes") and organism cultured is not related to an infection at another site AND Baby has at least 1 of the signs or symptoms enlisted (see "Notes") AND Physician institutes appropriate treatment (anti-staphylococcal penicillin or vancomycin) **3.** Pathological evidence of sepsis on autopsy *Other skin contaminants such as diphtheroids (*Corynebacterium* spp.), *Bacillus* (not *B anthracis*) spp., *Propionibacterium* spp., *Aerococcus* spp., *Micrococcus* spp.·were considered as contaminants In addition, the following were considered as contaminants: **1.** Organisms not fulfilling the definition of culture positive sepsis **2.** Three or more organisms in a single culture	Any one of the following symptoms/signs: Difficulty feeding Convulsions Movement only when stimulated Diarrhea (watery stools) Pus from umbilical stump Discharge (purulent) from ear Temperature (>37·5 °C or <36·5 °C) Heart rate (>180 min or <100 min) Respiratory rate (>60 min) Severe chest in-drawing Grunting Apnea CRT > 3 sec Cyanosis Lethargy/drowsiness Bulging fontanel Abdominal distension Multiple (>10) skin pustules Clinicians' discretion- (such as neonate not looking well, need for augmentation of respiratory support, etc.) Neonates were also subjected to sepsis work up if they had: maternal fever within 7 days before delivery or foul smelling liquor or prolonged rupture of membranes (>18 h)
Culture-negative sepsis (Possible bloodstream infection)	Baby has ALL of the following: **1.** Any one of the clinical sign/symptoms as enlisted above OR Existence of predisposing *risk factors*: maternal fever within 7 days before delivery or foul smelling liquor or prolonged rupture of membranes (>18 h) OR Radiological evidence of *pneumonia* OR Positive septic screen (see "Notes") **2.** Blood culture not done or no organisms detected in blood **3.** Physician institutes appropriate treatment for sepsis	Positive septic screen consists of two of the following parameters, namely total leukocyte count (TLC) <5 000 mm^3, absolute neutrophil count <1 500 mm^3, immature -to-total (IT) polymorph ratio of > 0·2, C-reactive protein >6 mg/dL, and micro-ESR > fall in mm more than 15 mm

<table>
<tr><td>Central-line associated blood stream infection (CLABSI)</td><td>Culture-positive bloodstream infections that occur when a central line or umbilical catheter was in place for 2 or more calendar days beforethe onset of the event</td><td>For calculating denominator, if more than one central line/umbilical catheter or either is in place, count only as a single device-day</td></tr>
<tr><td>Meningitis</td><td>This must meet ANY ONE of the following criteria
1. CSF culture is positive
AND
Baby has at least 1 of the signs or symptoms (as enlisted above)
2. If culture is negative, all of the following:
Any one of the clinical sign/symptoms listed above
AND
Any one of a–b:
A. Positive CSF examination with increased white cells, elevated protein, and/or decreased glucose
B. Positive gram stain for CSF
AND
If diagnosis is made antemortem, physician institutes appropriate antimicrobial therapy</td><td><table><tr><td>CSF components</td><td>Normal range</td></tr><tr><td>Cells/mm^3</td><td>Up to 30 cells</td></tr><tr><td>PMN (%)</td><td>60%</td></tr><tr><td>CSF protein (mg/dl)</td><td>Up to 150</td></tr><tr><td>CSF/blood glucose (%)</td><td>>60%</td></tr></table>(PMN: polymorphonuclear leukocytes; CSF: cerebrospinal fluid)</td></tr>
<tr><td>Necrotizing enterocolitis</td><td>This must meet ALL of the following:
1. At least two of these signs/symptoms: Pre-feed gastric aspirate of >50% of previous feed, abdominal distension, vomiting, bile/blood-stained aspirate
2. Bloody stools or occult blood in the stools
3. At least one of the following radiological evidence:
a. Pneumatosis intestinalis
b. Portal air
c. Free air under the diaphragm (pneumoperitoneum)
d. Unchanging “rigid” loops of small bowel</td><td></td></tr>
<tr><td>Urinary tract infection</td><td>This must meet ANY ONE of the following criteria:
1. A positive urine culture ≥10^5 microorganisms per cubic centimeter of urine with no more than two species of microorganisms
AND
At least 1 of the signs or symptoms (see “Notes”) with no other recognized cause
2. At least 1 of the signs or symptoms with no other recognized cause
AND
At least 1 of the following:
• Positive dipstick for leukocyte esterase and/or nitrate
• Pyuria (urine specimen with ≥10 WBC/mm^3 or ≥3 WBC/high-power fir ≥ of unspun urine)
• At least 2 urine cultures with repeated isolation of the same uropathogen (gram-negative bacteria or S</td><td>Symptoms: Fever, lethargy, vomiting, irritability/failure to thrive,
Signs: Temperature (>37·5 °C or <36·5 °C), heart rate (>180 min or <100 min), apnea

Culture reports of urine sample only collected by suprapubic method is to be included in this study (culture reports of urine samples collected by bag or catheterization are to be excluded for this study)·</td></tr>
</table>

	saprophyticus) with ≥10^2 colonies/ml in nonvoided specimens ≤10^5colonies/ml of a single uropathogen (gram-negative bacteria or *S saprophyticus*) in a patient being treated with an effective antimicrobial agent for a urinary tract infection • Physician institutes appropriate therapy for a urinary tract infection	
Systemic fungal infection	Baby has ANY ONE of the following criteria 1. Blood culture positive for yeasts AND Physician institutes appropriate therapy for a fungal infection 2. ≥2 of the risk factors (as mentioned adjoining in the notes) AND The presence of budding yeast/hyphae , either in cerebrospinal fluid or urine AND Physician institutes appropriate therapy for a systemic fungal infection	The main risk factors for systemic fungal infection (candidiasis) are as follows: i. Gestational age <32 wk ii. Previous fungal colonization (especially of the gastrointestinal tract) iii. Presence of central venous catheters iv. Prior use of parenteral nutrition and lipid emulsions v. Intubation time >7 days vi. Hospitalization >7 days vii. Shock or coagulopathy viii. Exposure to >2 antibiotics or any of third-generation cephalosporins ix. Exposure to systemic corticosteroids x. Exposure to H2 blocker xi. Exposure to theophyllines
Early-onset sepsis/infections	Onset of sepsis at or before 72 h of life (≤72h)	Includes sepsis occurring at 72 h also
Late-onset sepsis/infections	Onset of sepsis after 72 h of life (>72h)	
New episode of sepsis	When the neonate became symptomatic after 48 h of stopping appropriate antibiotic therapy or at clinician's discretion if new organism was cultured at fresh deterioration in an ongoing episode.	

Panel 1: Methodology additional details

Microbiology culture methods

- Blood was collected in Trypticase Soy broth (TSB; Diffco, India) and CSF in sterile screw cap vials. The specimen was transported to the research microbiology laboratory immediately after collection or kept in the onsite incubator maintained at 37^{o}C till transport.
- The organisms were identified by conventional culture methods at inborn units. They cultured specimens on 5% sheep blood agar (Biomerieux, France), chocolate agar and MacConkey agar (Oxoid, UK) and used standard microbiology methods for further processing, including antimicrobial susceptibility testing (AST).
- At outborn unit, the samples were processed using automated system -Bactec 9120/ Bactex FX 200 (Biomeriux, France). The bottles with positive growth were sub-cultured on the same sets of agar as above. The identification and AST of pathogen was performed using Vitek 2 compact system (Biomérieux, France).

Molecular analysis of resistance genes- was undertaken for *Klebsiella pneumoniae* and *Acinetobacter baumannii*.

- Phentotyping: The AST results of all *Klebsiella spp* isolates were confirmed by commercially available Etest (biomeriux, France) for meropenem and imipenem breakpoints (CLSI 2011-12). The resistant phenotypes were further tested for carbapenemase production by a) Modified Hodge test (MHT) and b) Metallo B Lactamases Etest (Biomeriux, France). Briefly, for MHT an overnight culture suspension of *E.coli* ATCC25922 adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of a Muller-Hilton agar plate (as per CDC guidelines). *K. pneumoniae* 1705 and *K. pneumoniae* 1706 were used for positive and negative control for clover leaf indentation for hodge test. The commercially available Etest MBL strip (BioMérieux, France) containing imipenem and imipenem EDTA was used. Reduction in the MIC of imipenem in the presence of EDTA was interpreted as positive. ATCC BAA 2146 and *Pseudomonas aeruginosa* ATCC were used as positive and negative controls respectively.
- PCR screening for carbapenemases: The bacterial DNA was extracted using Qiagen DNA mini kit. All carbapenem resistant isolates which were positive by MHT and /or MBL E-test were screened for the presence of *blaNDM*-1, *blaVIM, blaIMP* and *blaKPC* genes in multiplex PCR using primer by Laurent poirel et al 2011 (Webtable 2). The 25µl reaction mixture contained 1x PCR buffer (100mMtris /HCl+ 15mM MgCl2 and 50mMKCl,) 200mM of dNTPs, 20pm of each primer, 1U of taq polymerase (Bengalore Genei, India) and 50 ng of template DNA. The PCR amplified products obtained was purified using Axyprep gel cleanup kit (Axygen Biosciences, USA) and cleaned product was sequenced using 3130XL genetic analyser.
- DNA sequencing: The amplified product was purified using PCR clean up kit (Qiagen, Netherland) as per standard protocol. The purified sample was run on 3130XL genetic analyzer (Life technologies, USA). The deducted DNA sequence was aligned using Genedoc software (Nicholas and Nicholas, version 2.12) and uploaded on sequence database, which was further analysed with blast programme **(**http://www.ncbi.nlm.nih.gov/BLAST**).** The blast hits were checked, and the closest match score was recorded for the gene sequences. The final read were submitted in the gene database for generating accession number.

Risk factor analysis

- For analysis of risk factors of sepsis, we included the first episode of neonates with culture positive sepsis in the first 72 hours (for EOS) or between 73 hours and 28 days of life (for LOS) as 'cases', and those in whom sepsis was neither suspected nor diagnosed at any time in the first 28 days of life as 'controls' (Table 3). Only maternal and perinatal risk factors were considered for EOS. For LOS, only neonatal care practices such as duration of IV cannula, parenteral nutrition, or mechanical ventilation, and so on were included as 'antecedent' factors, achieved by truncating the information until the day before the onset of first episode of sepsis. Because of the non-linear relationship of the duration of neonatal care practices like IV cannula, mechanical ventilation, central line, etc. with LOS, we categorized most of the continuous variables into two or more strata (based on the adjusted odds ratio for each time-point) and then included them in the model.
- We first estimated the risk for each independent risk factor by logistic regression after adjusting for gestational age and birth weight. The variables that were found to be significant and clinically relevant in this analysis were then included along with gestational age and birth weight in the multivariate model−stepwise logistic regression with forward selection−to determine the final set of risk factors. The discriminatory power of the final model was evaluated using the area under the receiver operating characteristic curve.

Data analysis

- Statistical analysis was performed using Stata 11.2 (StataCorp, College Station, TX, USA) (Appendix). Incidence of sepsis was calculated by dividing the number of neonates with sepsis by the total number of NICU admissions. The incidence density was calculated as the number of episodes of sepsis per 1000 patient-days or 1000 device-days. Population attributable risk (PAR) of mortality for different categories of sepsis was estimated by using the command 'csi' in Stata.
- For analysis of risk factors, we included the first episode of culture-positive sepsis in the first 72 h (for EOS) or between 73 h and 28 days of life (for LOS) as "cases", and those in whom sepsis was neither suspected nor confirmed at any time in the first 28 days of life as "controls". Only maternal and perinatal risk factors were considered for EOS. For LOS, only neonatal care practices such as duration of IV cannula, parenteral nutrition, and mechanical ventilation were included as "antecedent" factors after truncating the information until a day before the onset of sepsis. We first estimated the risk for each independent risk factor by logistic regression after adjusting for gestational age and birth weight and study site. The significant and clinically relevant factors were then included along with gestational age and birth weight in the multivariate model—stepwise logistic regression with forward selection—to determine the independent risk factors. Because of the nonlinear relation between the duration of certain neonatal care practices such as IV cannula and LOS, we categorized these variables into two or more strata (based on the adjusted odds ratio for each time point) and then included them in the final model. The discriminatory power of the model was evaluated using the area under the receiver operating characteristic curve (AUC)

Table 2: Quality assurance measures

Clinical	• Prospective data collection (research nurses) and checking the case record forms (CRF) daily for accuracy and completion (research physician) • Cross-checking of randomly selected 20% CRF at each site were checked by the team form the coordinating centre (faculty investigator and research physician) on a weekly basis • Prospective assignment of final diagnosis by two pediatricians (faculty investigator and research physician)
Microbiology	• Uniform, specially procured high-quality culture media [5% sheep blood agar (BioMerieux, France) and McConkey agar (Oxoid, Hampshire, the UK)] and antibiotics disks • Cross-checking of antibiotic disk batches using ATCC strains on a regular basis (research microbiologist) • Validating identification and antibiograms of 10% isolates from each site at a different site (external quality assurance scheme; EQAS)
Data management	• Online double data entry in real-time at the sites (Data entry operators) • Discrepancies in double data entry resolved on an ongoing basis (research programmer) • Three-monthly data audits; errors resolved in consultation with site investigators (research biostatistician)
Overall	• Training of all research nurses off- and on-site using SOPs and videos (e.g., sample collection) initially and at periodic intervals (faculty investigator and research physician) • Dry run for four weeks before finalization of the CRF and SOPs • Weekly review meetings for assessing the progress

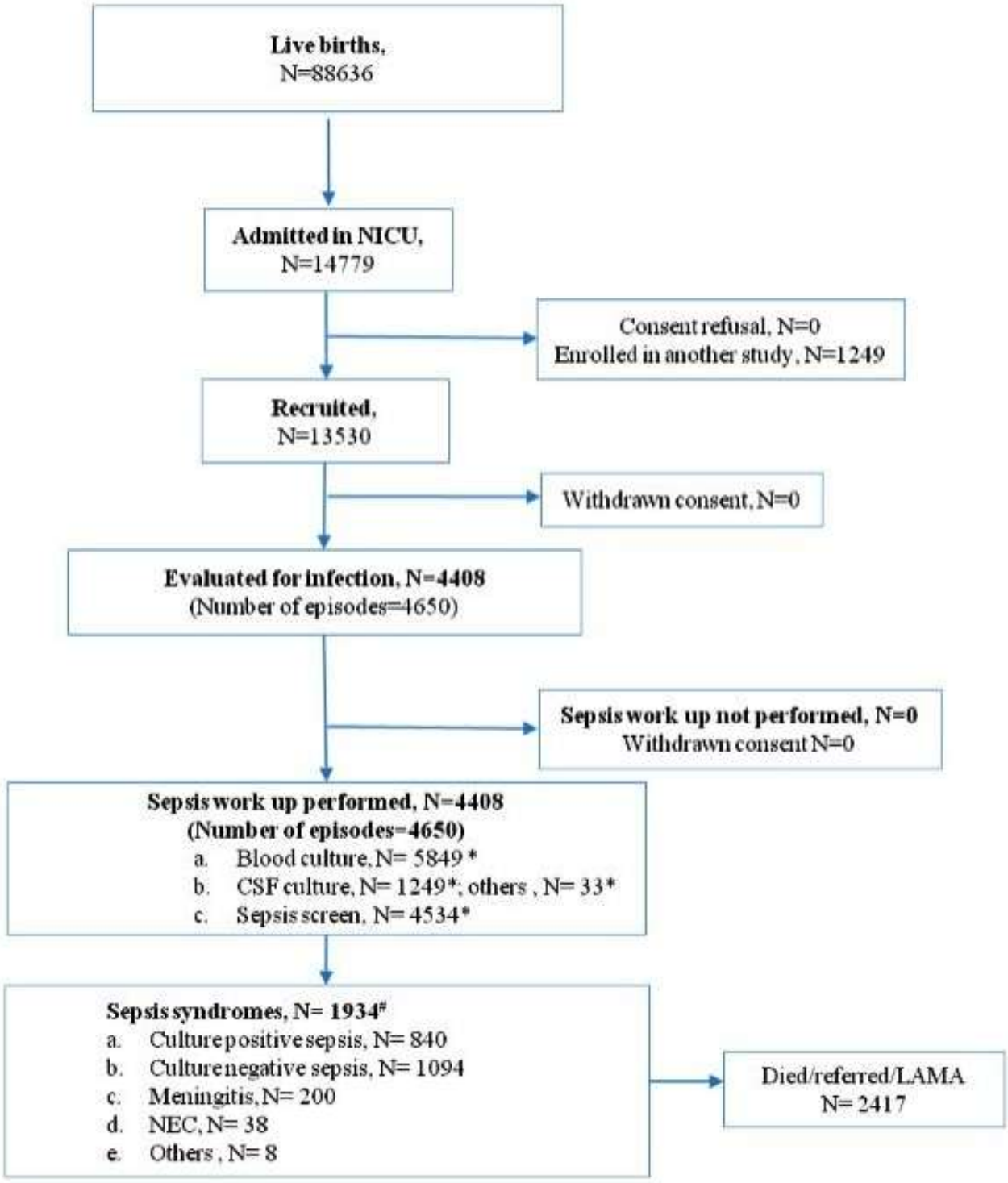

Figure 2: Study flow

Table 4: Inborn cohort- baseline details

Characteristics	(N = 13 530)
Birth weight, g	2211±741
Gestation, weeks	36·0±3·4
Small for gestation	3641 (26·9%)
Male gender	7678 (56·7%)
Multiple births	1493 (11·0%)
Caesarean delivery	4703 (34·8%)
Major malformations	804 (5·9%)
Positive pressure ventilation	4187 (31·0%)
Maternal/perinatal variables	
Antenatal corticosteroids	3970/4900 (81·0%)
Maternal fever within 7 days before delivery	1057 (7·8%)
Maternal antibiotics within 7 days before delivery **Received for 48h or more**	3170 (23·4%) 587 (4·3%)
Per vaginal examination (≥3)	5209 (38·5%)
Prolonged rupture of membranes (18 h or more)	1958 (14·5%)
Prolonged labour (24 h or more)	173 (1·3%)
Meconium-stained liquor	3073 (22·7%)
Foul-smelling liquor	374 (2·8%)
Neonatal care-related variables	
Intravenous fluids	8928 (66·0%)
Parenteral nutrition	272 (2·0%)
Peripheral arterial line	449 (3·3%)
Any central line	835 (6·2%)
Continuous positive airway pressure	2746 (20·3%)
Mechanical ventilation	1619 (12·0%)
Jaundice requiring phototherapy	3402 (25·1%)
Duration of NICU stay, days	2·7 (1·3-5·5)

Data expressed in mean±SD, median (IQR) or n (%); *only in neonates below 35-week gestation. NICU=neonatal intensive care unit

Table 5: Incidence and case fatality of neonatal sepsis- sitewise

	N	Total sepsis	Culture positive sepsis	Culture negative sepsis	Meningitis
Incidence*: number of neonates (%; 95% CI)					
Overall	13 530	1934 (14·3%; 13·8–14·9)	840 (6·2%; 5·8–6·6)	1094 (8·1%; 7·6–8·6)	200 (1·5%; 1·3–1·7)
Site 1	9239	1237 (13·4%; 12·7–14·1)	502 (5·4%; 5·0–5·9)	735 (8·0%; 7·4–8·5)	119 (1·3%; 1·1–1·5)
Site 2	2657	502 (18·9%; 17·4–20·4)	279 (10·5%; 9·4–11·7)	223 (8·4%; 7·4–9·5)	67 (2·5%; 1·9–3·2)
Site 3	1634	195 (11·9%; 10·4–13·6)	59 (3·6%; 2·7–4·6)	136 (8·3%; 7·0–9·8)	14 (0·9% 0·5–1·4)
Incidence density: number of episodes (density per 1000 patient-days; 95% CI)					
Overall	80 427	1980 (24·6; 23·6–25·7)	847 (10·5; 9·8–11·3)	1133 (14·1; 13·3–14·9)	200 (2·5; 2·2–2·8)
Site 1	42 419	1246 (29·4; 27·8–31·0)	502 (11·8; 10·8–12·9)	744 (17·5; 16·3–18·8)	119 (2·8; 2·3–3·3)
Site 2	21 342	517 (24·2; 22·2–26·4)	281 (13·1; 11·6–14·7)	236 (11·1; 9·7–12·5)	64 (3·0; 2·3–3·8)
Site 3	16 666	217 (13·0; 11·3–14·8)	64 (3·8; 2·9–4·9)	153 (9·2; 7·8–10·7)	14 (0·8; 0·4–1·4)
Case fatality rate: number of neonates (%; 95% CI)					

Overall	–	496/1934 (25·6%; 23·7–27·7)	400/840 (47·6%; 44·2–51·0)	96/1094 (8·8%; 7·2–10·6)	102/200 (51·0%; 43·8–58·1)
Site 1		248/1237 (20·0%; 17·8–22·4)	200/502 (39·8%; 35·5–44·3)	48/735 (6·5%; 4·8–8·6)	45/119 (37·8%; 29·1–47·2)
Site 2	-	226/502 (45·0%; 40·6–49·5)	188/279 (67·4%; 61·5–72·8)	38/223 (17·0%; 12·3–22·6)	56/67 (83·5%; 72·5–91·5)
Site 3	-	22/195 (11·3%; 7·2–16·6)	12/59 (20·3%; 11·0–32·8)	10/136 (7·3%; 3·6–13·1)	1/14 (7·1 %; 0·2–33·8)

Data expressed as n (%); * among those admitted in the NICUs

Table 6 Pathogen profile by site

Organism name	Overall (n=1005)	Site 1 (n=576)	Site 2 (n=359)	Site 3 (n=70)
***Acinetobacter* spp.**	222 (22·1%)	155 (26·9%)	62 (17·3%)	5 (7·1%)
***Klebsiella* spp.**	169 (16·8%)	67 (11·6%)	89 (24·8%)	13 (18·6%)
CoNS	150 (14·9%)	90 (15·6%)	28 (7·8%)	32 (45·7%)
E. coli	137 (13·6%)	64 (11·1%)	69 (19·2%)	4 (5·7%)
Staphylococcus aureus	122 (12·1%)	88 (15·3%)	29 (8·1%)	5 (7·1%)
***Pseudomonas* spp.**	68 (6·8%)	10 (1·7%)	50 (13·9%)	8 (11·4%)
***Enterococcus* spp.**	56 (5·6%)	33 (5·7%)	22 (6·1%)	1 (1·4%)
***Enterobacter* spp.**	44 (4·4%)	41 (7·1%)	2 (0·6%)	1 (1·4%)
***Streptococcus* spp.**	12 (1·2%)	10 (1·7%)	2 (0·6%)	0
GBS	8 (0·8%)	8 (1·4%)	0	0
***Candida* spp.**	7 (0·7%)	4 (0·7%)	2 (0·6%)	1 (1·4%)
Others	10 (1·0%)	6 (1·0)	4 (1·1%)	0

NB1: For multiple isolates detected from a single episode, we used the following rules:
• If single culture was sent and it grew two organisms, both organisms were included.
• If multiple cultures were sent, and same organism was isolated in all, only the first organism was included.
If multiple cultures were sent, and different were organisms isolated, all organisms were included.
NB2: The case fatality rate (CFR) in neonates with sepsis due to Gram-negative pathogens was higher than that of neonates infected with gram-positive pathogens (58•7% vs. 32•8%). Neonates infected with Pseudomonas spp. and E coli had the highest CFR –76•8% and 60•9%, respectively.

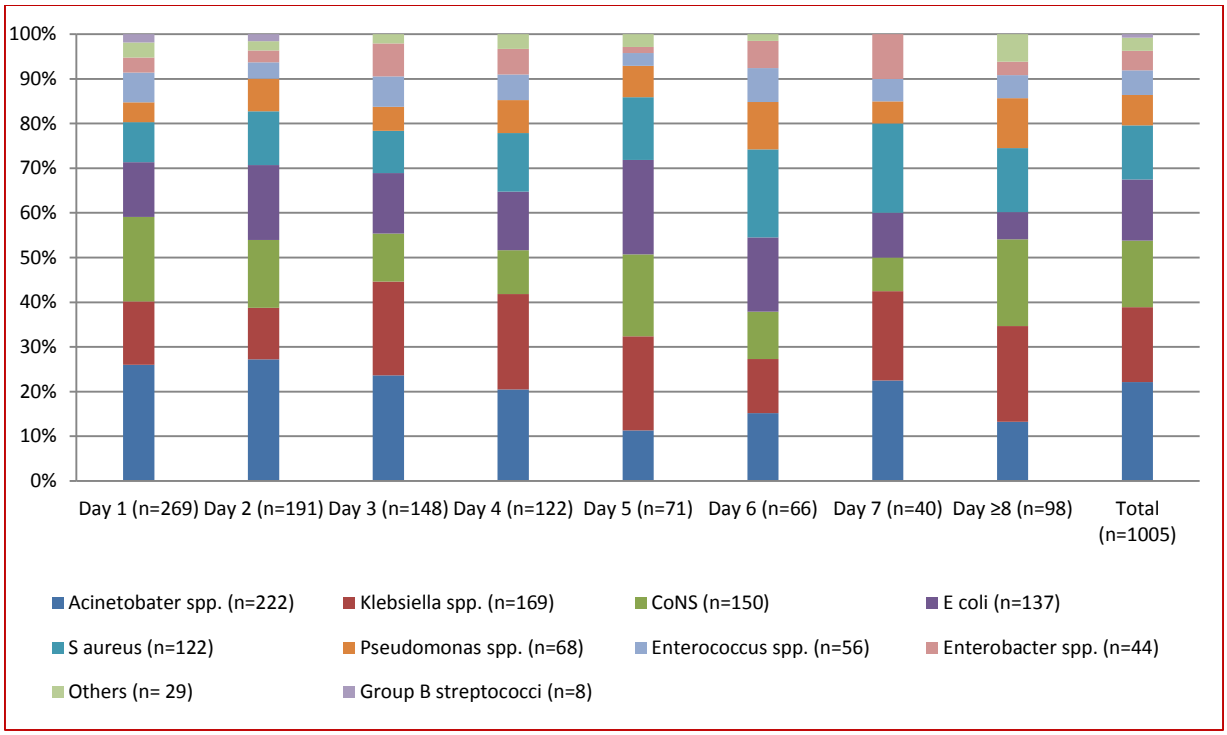

Figure 3: Variation in the distribution of isolates by age (day since birth)

Table 7: AMR pattern of Gram-negative isolates- Inborn cohort

	Acinetobacter spp. (N= 222)	*Klebsiella* spp. (N= 169)	*E coli* (N=137)	*Pseudomonas* spp. (N=68)	*Enterobacter* spp. (N=44)
Amoxicillin	180/195 (92·3%)	154/158 (97·4%)	116/131 (88·5%)	53/55 (96·4%)	36/38 (94·7%)
Co- amoxiclav.	178/199 (89·4%)	138 /157 (87·9%)	98/132 (74·2%)	53/55 (96·4%)	37/38 (97·4%)
Amikacin	180/214 (84·1%)	75 /165 (45·4%)	31/137 (22·6%)	11/66 (16·7%)	15/42 (35·7%)
Cefotaxime	95/102 (93·1%)	116/150 (77·3%)	75/117 (64·1%)	47/52 (90·4%)	26/35 (74·3%)
Ceftazidime	179/205 (87·3%)	114/158 (72·1%)	73/126 (58·0%)	26/66 (39·4%)	26/38 (68·4%)
Ceftriaxone	75/ 82 (91·5%)	74/104 (71·1%)	51/76 (67·1%)	32/47 (68·1%)	1/3 (33·3%)
Cefoperazone+sublactam	151/217 (69·7%)	67/162 (41·4%)	29/132 (22·0%)	10/65 (15·4%)	17/41 (41·6%)
Ciprofloxacin	181/217 (83·4%)	84/166 (50·6%)	77/135 (57·0%)	14/66 (21·2%)	23/42 (54·7%)
Colistin	2/210 (1·0%)	1/160 (0·6%)	3/133 (2·6%)	1/66 (1·5%)	0/39
Gentamicin	171/206 (83·0%)	102/164 (62·2%)	53/135 (39·3%)	13/62 (21·0%)	26/41 (63·4%)
Imipenem	167/215 (77·7%)	58/166 (35·0%)	19/137 (13·8%)	19/66 (28·8%)	8/42 (19·0%)
Meropenem	173/215 (80·5%)	59/166 (35·5%)	19/137 (13·8%)	18/66 (27·2%)	7/42 (16·7%)
Netilmicin	134/208 (64·4%)	95/165 (57·6%)	39/136 (28·7%)	15/66 (22·7%)	22/42 (52·4%)

Piperacillin + tazobactam	179/213 (84·0%)	91/167 (54·5%)	49/137 (35·7%)	11/66 (16·7%)	21/41 (51·2%)
Tigecycline	50/210 (23·8%)	9/157 (5·7%)	3/129 (2·3%)	41/57 (72·0%)	0 /41

Data expressed in n/N (%); there are variations in denominators in each cell as antibiotics sensitivity testing for all drugs was not done. NB: Antibiotics tested for Gram negative pathogens:Amoxycillin, Co-amoxyclav, Cefotaxime, Ceftazidime, Ceftriaxone, Gentamicin, Amikacin, Netilmycin, Ciprofloxacin, Piperacillin+tazobactam, Cefoperazone +Sulbactum, Meropenem, Imipenem, Ertapenam, Doripenem, Tigecycline, Colistin

Table 8: AMR pattern of Gram-positive isolates- Inborn cohort

	Coagulase-negative staphylococci (N=150)	***Staphylococcus aureus* (N=122)**	***Enterococcus* spp.* (N=56)**
Methicillin	85/142 (59·8%)	43/116 (37·1%)	-
Amikacin	19/139 (13·7%)	2/112 (1·7%)	-
Amoxicillin	119/143 (83·2%)	102/112 (91·1%)	23/49 (47·0%)
Co-amoxiclav	69/136 (50·7%)	61/106 (57·5%)	-
Cefazolin	45/111 (40·5%)	25/84 (29·8%)	-
Ciprofloxacin	87/140 (62·1%)	81/116 (69·8%)	41/51 (80·4%)
Clindamycin	48/140 (34·3%)	20/116 (17·2%)	-
Gentamicin	67/139 (48·2%)	29/113 (25·7%)	25/50 (50·0%)
Linezolid	3/116 (2·6%)	0/99	3/52 (5·8%)
Teicoplanin	6/140 (4·3%)	1/115 (0·9%)	14/51 (27·4%)
Vancomycin	0/140	0/116	13/50 (26·0%)

Data expressed in n/N (%); there are variations in denominators in each cell as antibiotics sensitivity testing for all drugs was not done. * Gentamicin 120 microgram disks were used for the antibiogram of enterococci; other antibiotics (indicated by "-") were not tested. NB: Antibiotics tested for Gram positive pathogens- Amoxycillin, Cefazolin, Co-amoxyclav, Ciprofloxacin, Gentamicin, Amikacin, Netilmycin, Vancomycin, Teicoplanin, Linezolid, Co-trimoxazole, Clindamycin, Erythromycin, Tigecycline, Cefoxitin (-for methicillin)

Table 9: Sepsis categories and their attributable and population attributable risk of mortality

Categories	**Died (n=2103)**	**Survived (n=85033)**	**Relative risk (95% CI)**	**Attributable risk percentage (AR%)**	**Population attributable risk (PAR)**
No sepsis	1457 (69·3%)	83817 (98·6%)	1·0	-	-
Culture negative sepsis	158 (7·5%)	894 (1·0%)	8·79 (7·55 to 10·2)	88·6% (86·7 to 90·2)	**8·6%**
Culture positive sepsis by '*sensitive*' organisms (MDR-/cefoxitin-sensitive)	207 (9·8%)	165 (0·2%)	32·6 (29·34 to 36·13)	96·9% (96·6 to 97·2)	**12·0%**
Culture positive sepsis by '*resistant*' organisms (MDR+/cefoxitin-resistant)	281 (13·4%)	157 (0·2%)	37·5 (34·43 to 40·94)	97·3% (97·1 to 97·5)	**15·7%**

'Resistance' defined as multidrug resistant (for Gram-negative organisms) OR cefoxitin resistant (for Gram-positive organisms); NB: For the purpose of calculating PAR, we used all live births as the denominator and assumed that the remaining neonates who did not get admitted in NICU (and therefore not enrolled in the study) did not develop sepsis.

Table 10: Risk factors of early-onset culture-positive sepsis (multivariate analysis)

Characteristic	Culture-positive sepsis (N = 523)	No sepsis (N = 9 714)	Adjusted OR (95% CI)
Gestation (wk)	33·7±4·2	36·4±3·0	0·80 (0·78–0·81)
Number of antenatal care (3 visits or more)	185 (35·4)	3 748 (38·6)	1·34 (1·09–1·63)
Male gender	303 (58·0)	5 475 (456·4)	1·19 (1·10–1·29)
Prolonged rupture of membranes (18h or more)	102 (19·5)	801 (8·2)	1·91 (1·09–1·63)
Meconium-stained liquor	111 (21·2)	2 258 (23·2)	1·80 (1·43–2·27)
Major malformations	30 (5·7)	475 (4·9)	1·55 (1·27–1·88)
Caesarean delivery	135 (25·8)	3 470 (35·7)	0·72 (0·55–0·94)

Values expressed in mean±SD *or n* (%)

Table 11: Risk factors of late-onset culture-positive sepsis (multivariate analysis)

Characteristic	Culture-positive sepsis (*N* = 317)	No sepsis (*N*= 9 319)	Adjusted OR (95% CI)
Gestation (wk)	33·9±4·3	36·5±2·8	0·9 (0·8–1·0)
Birth weight (g)	1 796·8±769·1	2 296·5±695·4	0·9 (0·9–1·0)
Male gender	185 (58·4)	5 248 (56·3)	1·2 (1·1–1·3)
Intravenous fluids			
None	43 (13·6)	4 600 (49·4)	1·0
<48 h	69 (21·8)	3 262 (35·0)	2·5 (1·8–3·6)
>48 h	205 (64·7)	1 457 (15·6)	11·6 (9·1–14·9)
Mechanical ventilation			
None	227 (71·6)	9 099 (97·6)	1·0
<48 h	33 (10·4)	138 (1·5)	5·5 (3·4–8·9)
>48 h	57 (18·0)	82 (0·9)	27·0 (10·5–69·2)

Values expressed in *n* (%) or mean±SD. OR = odds ratio

Outborn Cohort

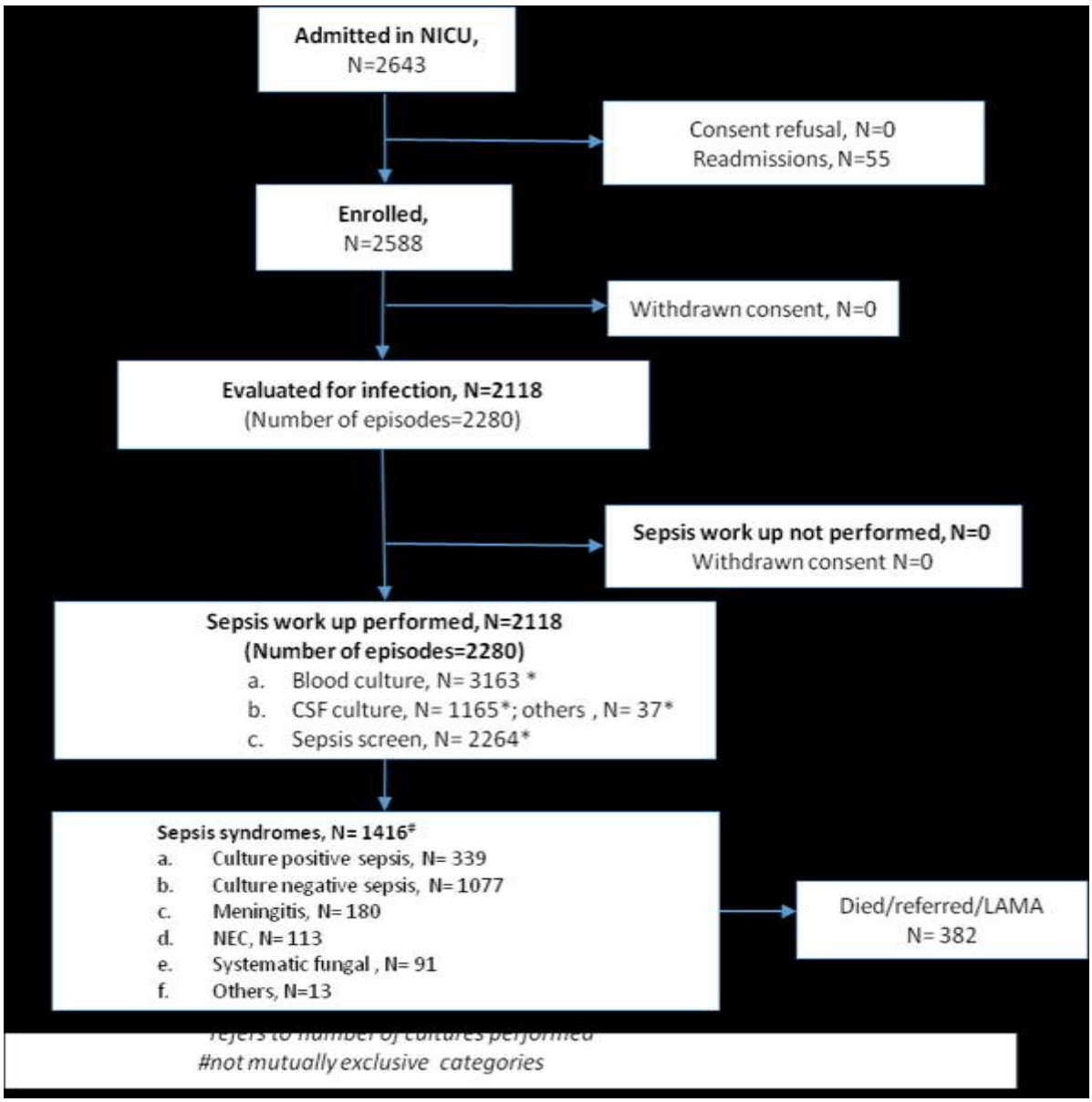

Figure 4: Study flow

Table 12: Demographic characteristics of enrolled infants

Items	Values (n =2588)
Birth weight, *g* (n= 2058)	2204±731
Gestational age, week (n=2583)	35.4±2.8
Male gender	1680 (64.9%)
Age at admission, days	5 (2-11)
Maternal details	
Urinary tract infection in last trimester	208/2287 (9.09%)
Fever within 7 days prior to delivery	211/2303 (9.2%)
Antibiotics within 7 days prior to delivery	142 (5.5%)
Antenatal steroids (in <35 wk gestation; n/N)	102/701 (14.5%)
Vaginal examinations (≥3)	702/2281 (30.8%)

Rupture of membranes (>18h)	397/2577 (15.4%)
Meconium stained liquor	364/2571 (14.2%)
Foul smelling liquor	53/2550 (2.1%)
Birth details	
Caesarean delivery	575/2580 (22.3%)
Home delivery	550/2588 (21.2%)
Delivery by traditional birth attendant	456/2306 (19.8%)
Did not cry at birth	643/2577 (24.9%)
Resuscitation at birth	182/2273 (8.01%)
Unhygienic cord practices (application of oil, cow dung, etc.)	879/2544 (34.5%)
Pre-lacteal feeds	682/2307 (29.6%)
Previous hospitalization of the neonate (n=984)	
Healthcare facility **Primary/secondary level government hospital** **Tertiary level government hospital** **Private hospital**	 56 (5.7%) 198 (20.1%) 730 (74.2%)
Duration of stay, days	2 (1 to 7)
Mechanical ventilation	271/984 (27.5%)
Antibiotic therapy **Duration of antibiotic therapy, days**	825/984 (83.84%) 3 (1 to 6)
Type of antibiotics **Amikacin** **Cefotaxime** **Meropenem** **Piperacillin-tazobactam** **Colistin**	 598 (72.5%) 446 (54.1%) 139 (16.8%) 116 (14.1%) 11 (1.3%)
Therapeutic modalities at study hospital (n=2306)	
Intravenous fluids	1950 (84.6%)
Parenteral nutrition	1415 (61.4%)
Central catheters*	820 (35.6%)
Intermittent mandatory ventilation	653 (28.3%)
Blood/plasma transfusion	461 (20.0%)
Continuous positive airway pressure (CPAP)	319 (13.8%)
Corticosteroids	310 (13.4%)
Antibiotic therapy **Duration of antibiotic therapy**	2243/2588 (86.7%) 6 (4 to 12)
Duration of NICU stay, days	6 (3 to 1

Data expressed as no (%), median (IQR) or mean±SD

*include umbilical venous/arterial catheter or peripherally inserted central catheter

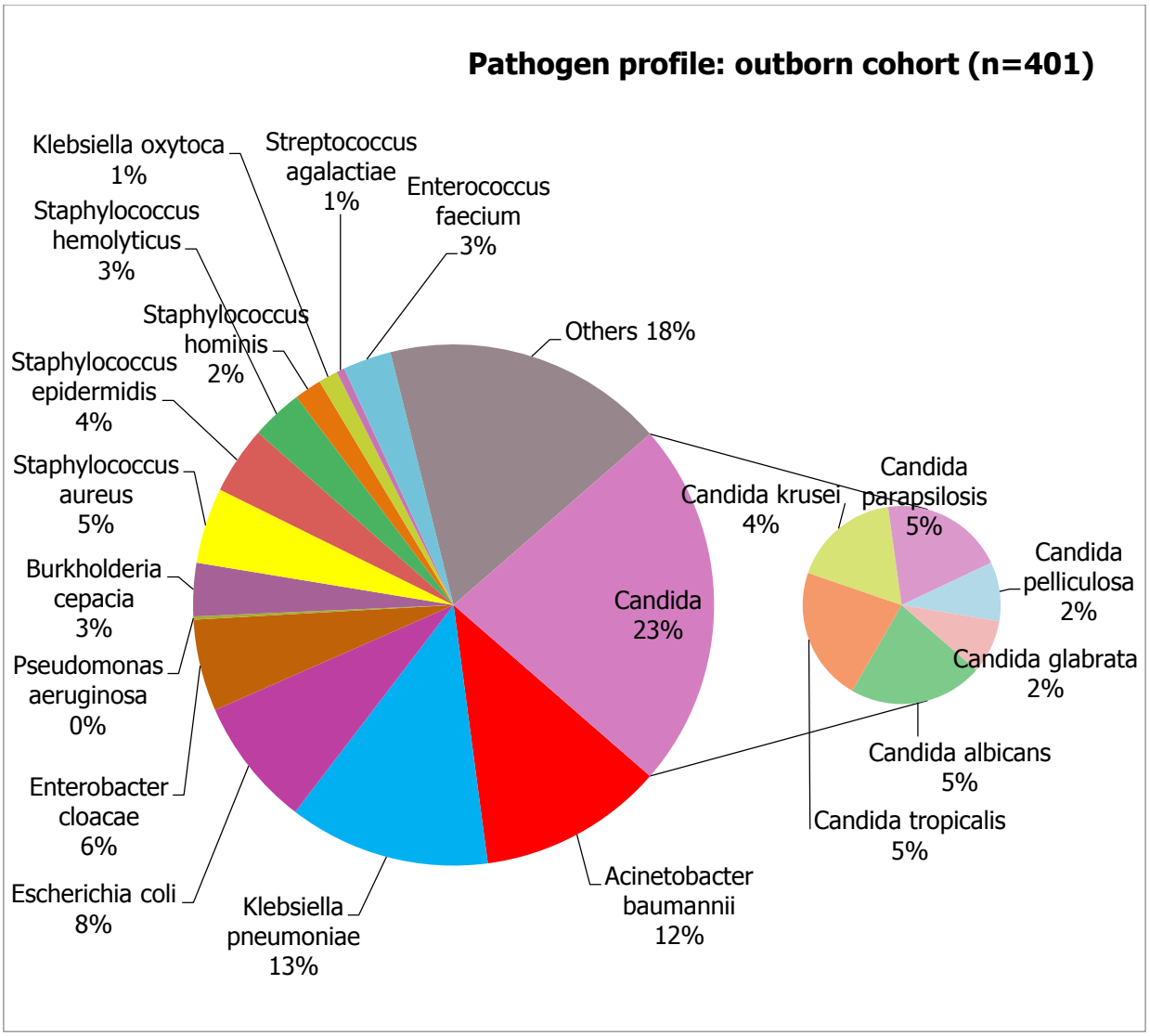

Figure 5: Pathogen profile in outborn cohort

Panel 2: Systemic fungal infections in outborn cohort

Invasive Systemic fungal infections: Among the 90 neonates diagnosed with fungal infections, the mean (SD) birth weight and gestation were 1751 (698) g and 33.8 (4) weeks, respectively. Around two-thirds of these neonates were born at or after 32 weeks of gestation (n=67, 73.3%) and had a birth weight of 1500 g or more (n= 49, 61.5%). The median (IQR) age at admission was 7.2 (3.7-15.6) days. Most (90.2%) were diagnosed within 12 hours of admission to index hospital (median [IQR] of admission-to-detection interval 0 [0-1] h). Three-fourths of neonates (n=66, 73.3%) were hospitalized previously, the median duration of stay being 6.5 (4-14) days. Almost all of them (n=62) had received broad-spectrum antibiotics including cephalosporins (50.0%), meropenem (32.3%), piperacillin-tazobactam (16.1%), and colisitin (9.7%). Over 40% (27/62) had received mechanical ventilation (median [IQR]: 5 [2-8] days) during their prior hospital stay.

Only one of the tested isolates (Candida sphaerica) was resistant to fluconazole and voriconazole. Four isolates (Candida krusei (n=2) and Candida guilliermondii (n=2)) were resistant to Amphotercin B.

Table 13: Pathogen specific case fatality rates

Pathogen	**Overall**
Acinetobacter baumannii	17/46 (37.0%)
Klebsiella pneumoniae	10/50 (20.0%)
E coli	13/32 (40.6%)
Enterobacter cloacae	3/23 (13.0%)
Enterococcus faecium	3/12 (25.0%)
Staphylococcus aureus	3/19 (15.8%)
Staphylococcus epidermidis	2/17 (11.8%)
Staphylococcus hemolyticus	3/13 (23.0%)
Staphylococcus hominis	1/7 (14.3%)
Candida albicans	5/20 (25.0%)
Candida tropicalis	3/20 (15.0%)
Candida krusei	3/16 (18.7%)
Candida parapsilosis	6/18 (33.3%)
Candida pelliculosa	2/9 (2.20%)
Candida glabrata	2/8 (25.0%)
Other *Candida* spp	4/10

Table 14: Phenotyping/ Genotyping summary for Carbapenem resistant *Klebsiella pneumoniae*

Study site	***Klebsiella* isolated**	**Carbapenem Resistant *Klebsiella* (CRK)**	**Phenotyping for Carbapenem Producers Genotyping for Carbapenem producers***		
			MHT positive	MBL E test positive	NDM-1 PCR
Site 1	93	38	4/38 (10.5%)	31/38 (81.5%)	29/38 (76.3%)
Site 2	75	38	3/38 (7.8%)	35/38 (92.1%)	33/38 (86.8%)
Site 3	9	4	1/4 (25%)	3/4 (75.0%)	2/3 (66.6%)
Site 4	38	26	2/26 (7.6%)	25/26 (96.1%)	22/26 (84.6%)
Total	215	106	10/106 (9.4%)	94/106 (88.6%)	86/106(81.1%)

*Genotyping for carbapenem producers included screening for VIM, IMP and KPC genes also, No isolate amplified these three gene

Table 15: Accession no of CRK and CRA

SN	Isolate	Accession number
1	Carbapenem resistant *Klebsiella pneumoniae*	KP036457, KP036458, KP036459, and KP036460
2	Carbapenem resistant *Acinetobacter* spp.	NDM-1: KR476385-KR476386, Oxa-23: KT721905, Oxa-51: KT721906, Oxa-58: KT721907, Imp gene: KT721908

Table 16: Genotyping profile for CRAB

Site	*Acinetobacter* isolated	Carbapenem resistant *Acinetobacter* (CRAB)	Processed for Molecular analysis	Genotyping for carbapenemase producers					
				NDM-1	*VIM*	*IMP*	*OXA 51*	*OXA 58*	*OXA 23*
Site 1	90	73	50	19 (38%)	0	0	50 (100%)	0	35 (70%)
Site 2	5	1	1	0	0	0	1 (100%)	1 (100%)	1 (100%)
Site 3	245	203	203	44 (22%)	0	7 (3.5%)	203 (100%)	24 (12%)	193 (95%)
Site 4	46	39	39	7 (18%)	0	0	39	4 (10.2%)	29 (74.3%)
Total	386	317	294 (92%)	70 (24%)	0	7 (2.38%)	293 (100%)	29 (10%)	258 (87.7%)

PROJECT 2

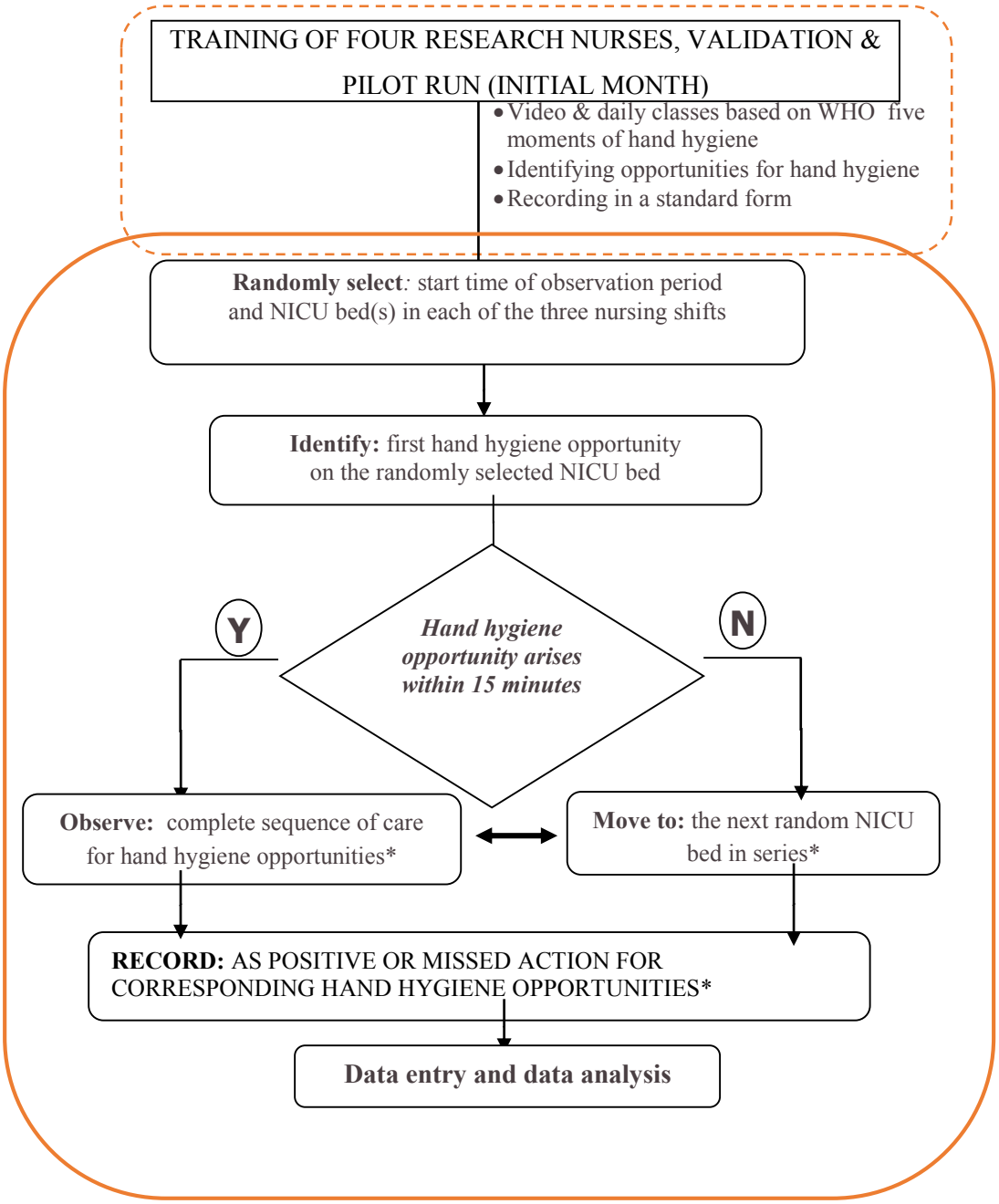

Figure 6: Study plan (*one or more of the steps repeated to record a maximum of 24 opportunities in a single observation period)