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Original Article

Paediatrics Section

Community Acquired Neonatal Sepsis- A Cohort Study in Rural Southern India

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ABSTRACT

Introduction: Neonatal sepsis is responsible for 30% to 50% of all neonatal deaths in developing countries in a year. Incidence of neonatal sepsis was reported to range between 30.7-35.9 per 1000 live births in low middle income African and Southeast Asian countries and 23% neonatal deaths occur due to neonatal sepsis.

Aim: To identify the organisms isolated from various surfaces around the neonate, including body surfaces of neonate and mother, in a hospital and home environment and to estimate the incidence of Community Acquired Neonatal Sepsis (CANS) among hospital-born neonates.

Materials and Methods: A cohort study was conducted in a rural area of Southern India, enrolling 100 healthy neonates, born in a rural tertiary care hospital. These neonates were then followed-up in community. Knowledge regarding antenatal and postnatal practices related to neonatal sepsis prevention was assessed. Swabs were collected from various surfaces, on the day of birth (day 1) in hospital, on day 3 and day 7 of life of the neonate, at household. The outcome measure was CANS. The data was collected by trained investigators and swabs were transported to the lab under aseptic conditions as per the standard protocol. Descriptive analysis by calculating frequencies and means were done and the data were presented as rates, proportions and mean±standard deviation.

Results: Total 100 newborns were included for the analysis; 49% were males and 51% had low birth weight. Forty two neonates contracted infection in their household, of which 32 were hospitalised; none were culture confirmed sepsis. The CANS (culture negative) rate was 3.23 per 1000 live births (95% CI:0.00-4.28; p=0.01). The common organisms isolated from different surfaces in hospital were coagulase negative Staphylococci and Klebsiella, while *E.coli*, Citrobacter, Acinetobacter, Pseudomonas and non-ferementers were additionally isolated from household surfaces. Caretaker's hands showed the highest number of organisms, both in hospital (26%) and in community (12%).

Conclusion: Institution born neonates followed in households showed a high prevalence (60.4%) of pathogenic organisms from various surface swabs both in institution (83%) and household (54.5%). Most common organisms isolated from different surfaces in hospital as well as home were coagulase negative Staphylococci, Klebsiella and *E. coli*. *Staphylococcus aureus* was highly prevalent in hospital compared to home.

Keywords: Antenatal, Knowledge, Neonate, Newborn care practices, Surface organisms

INTRODUCTION

Neonatal mortality accounts for nearly half of the global child mortality, with a reported five million neonatal deaths occurring annually [1]. About one million deaths caused due to infections, predominantly sepsis, are concentrated in developing countries [2]. World Bank data indicated India having a neonatal mortality rate of 27.7 per 1000 live births in 2015, about 50% of which was attributed to sepsis. Even as the rate of institutional deliveries in India stands high at 79%, neonates born in hospital are usually discharged soon after birth [3,4]. Most studies measuring the burden of neonatal morbidities have focused on asphyxia or sepsis in hospitals which do not provide true estimates since the results cannot be extrapolated to community settings [5-7]. Few population-based studies have reported clinical sepsis rates ranging from 49 to 170/1000 live births in rural India while a greater burden remains to be unearthed [8-12].

Neonatal sepsis is classically defined as the presence of symptoms of sepsis in the neonatal period combined with bacteriological isolation of an infectious agent from blood or Cerebrospinal Fluid (CSF) [13]. It is classified into 'early-onset' if occurring within the first seven days of life and 'late-onset' if it occurs after this time, therefore presenting a wide array of associated risk factors. Aetiological research has implicated the roles of different pathogens such as Gram negative, Gram positive bacteria e.g., Klebsiella, *E. coli*, pseudomonas, *Staph. aureus*, Acinetobacter, *Streptococcus pneumoniae*, Group B streptococci, and others,

as well as classical predisposing risk factors for infection in neonates that include low birth weight, prematurity, prolonged rupture of membranes and a long delivery period, insufficient weight gain, and inappropriate breastfeeding practices [4,14,15]. Social factors like poor care seeking behaviours for infant's health, poorer sanitation, decrease in access to healthcare facilities, women having a low social standing and lack of autonomy also have an impact on infant's sepsis. Additionally, child gender bias has been shown to play a strong role in routine care and health care seeking for sick children [16].

Few studies have explored the knowledge and child rearing practices of women, reporting lack of knowledge and faulty practices. A longitudinal study following women from pre-pregnancy to childbirth and beyond was undertaken in a rural area in South India [17]. The verbal autopsies of neonatal deaths studied in this project revealed the maximum number of deaths caused due to sepsis (63%), typically community acquired, as well as other infectious diseases (26%). This preliminary information asserts need to study the true rural incidence of sepsis and deeper knowledge of contributing risk factors. Till date no study exists from Southern Indian state of Telangana that has identified the common aetiologic agents other than behaviour.

The primary aim of the study was to report the organisms isolated from various surfaces which come in contact with the neonate in hospital and home, including body surface of neonate and mother and to estimate the incidence of CANS in rural area of Telangana, India.

MATERIALS AND METHODS

Study Design and Participants

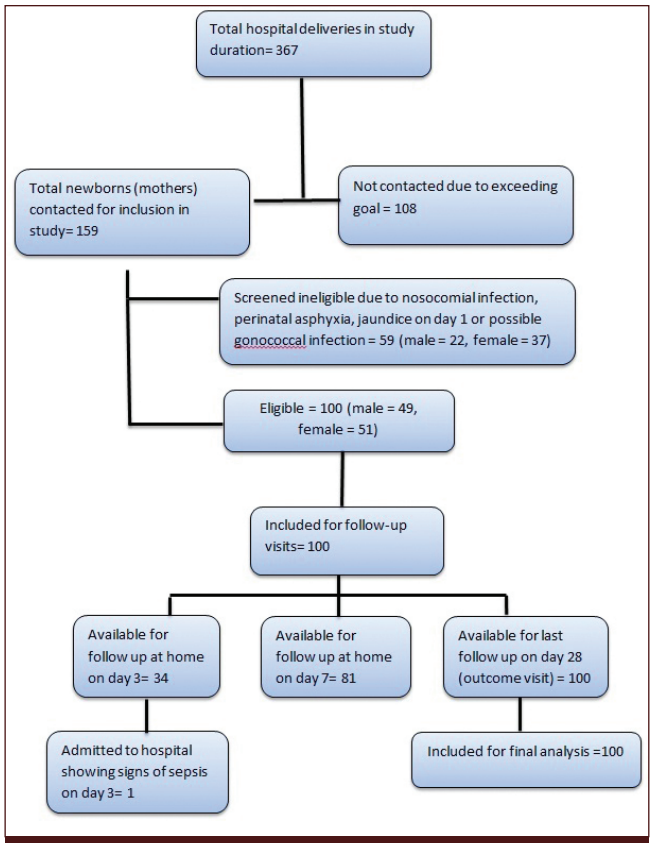
A cohort study was implemented for a representative sample of 100 newborns delivered at the Mediciti Institute of Medical Sciences (MIMS), a 500-bed teaching hospital, located in a rural area in Telangana state of Southern India. The study recruited newborns between 1st August 2017 to 30th September 2017 and followed them up till the last recruited newborn completed 28 days of life. Ethical approval (No. EC/2K17/ dated 21.04.17) was obtained from Institutional Review Board (IRB) of Mediciti Institute of Medical Sciences (MIMS), Hyderabad and approved by Indian Council of Medical Research (ICMR), New Delhi. The MIMS hospital provides tertiary care services for a population of about 43,270 in 40 villages in the Medchal district on the Northern outskirts of Hyderabad.

Sample Size and Power

The sample size of 100 newborns was chosen to accurately estimate incidence of neonatal sepsis, assuming a CANS prevalence of 6.03% [15] and acceptable level of error at 5%; a sample size of 90 neonates was found sufficient to report neonatal sepsis incidence with 80% power, to which a non-response rate of 10% was added.

Recruitment Process

A two-member team went to the labor room of Mediciti hospital to identify eligible mothers likely to give birth within 24 hours. Recruitment was conducted three days per week (Monday, Thursday and Saturday). The goal was to recruit two newborns during each visit that were to be followed-up in home on pre-decided days, up to 28 days of life. Telugu translated and IRB approved consent form, recruitment script and eligibility form were developed. Eligible newborns and consenting mothers were included for baseline data collection and scheduled for the follow-up visits at home [Table/Fig-1].



[Table/Fig-1]: Recruitment flowchart for the study.

Eligibility

Newborns were eligible to be included if they were delivered at Mediciti Hospital, apparently healthy and lived in any of the 40 villages in Medchal mandal. Eligibility also included the mother to give informed consent, and no plan to move from the area over the next 28 days. Newborns having congenital anomalies detected within a day of birth, history of perinatal asphyxia, having jaundice on the first day of life and possible gonococcal eye infection were excluded. In addition, those who had signs of infection while in hospital were excluded from further follow-up. Nosocomial infections were excluded by excluding sample collection from following: (1) neonates admitted for three days in hospital after birth; (2) neonates who developed symptoms of

infection at three days of life while still in hospital; (3) neonates who had invasive procedures (IV insertion, intubation, etc.,) while in hospital; and (4) neonates who consulted within seven days of discharge.

Data Collection

Assessment for the present study was organised into one baseline hospital visit on first day of life, and three follow-up home visits thereafter on day 3, 7 and 28. The duration of each visit was approximately seven hours. The hospital visit consisted of anthropometry and clinical examination of the newborn and collection of swabs from the newborn, mother and environment; and an interview of the mother including questions on demographic information, socio-economic status, child care knowledge and behaviours, and antenatal health and exposures. The second and third visit on day 3 and 7 of neonate's life respectively at the neonate's home consisted of collections of swabs of neonate, mother and environment, detailed clinical examination of the neonate, anthropometry and interview of the mother to record change in knowledge and practices. An environmental audit was also done during these visits. On the final visit on day 28 of the newborn's life, data pertaining to survival and outcomes status of the neonate, anthropometry and clinical examination of the neonate was done. This visit was also utilised to impart knowledge to the mother about infant care and risk factors, nutrition, family planning advice and environmental sanitation. All forms and protocols were modeled after major global CANS studies [8,15]. The rationale for the measures chosen was based on a comprehensive model of major common newborn conditions and antenatal parameters, assessed with objective measures. The data collection and questionnaire plan are summarised in [Table/Fig-2].

Components of baseline assessment	
Measurement category	**Data points, procedures and instruments**
First visit (Day 1 of life, in hospital)	
Interviewer administered questionnaires for mother	
General characteristics	Age, religion, caste of mother and father
Socioeconomic and lifestyle	• Education of mother and father • Occupation of mother and father • Family income • Family size
Maternal characteristics	• Age at marriage • Age at first pregnancy • Current parity • Number of antenatal check-ups received for present pregnancy • Gestational age at first check-up • No. of Iron tablets received and consumed • Tetanus toxoid received • Dietary history • Presence of danger signs during pregnancy
Maternal knowledge (self-report of mother's knowledge)	• Danger signs during pregnancy including convulsions, high blood pressure, oedema, excessive bleeding, reduced foetal movements, high grade fever. • Preferred health facility for treatment • Birth preparedness • Benefits of hospital delivery • Complications during labour • Preferred facility for treatment of complications • Knowledge regarding newborn health including birth weight of child, danger signs in newborns, prevention of hypothermia, prevention of infections, prevention of diarrhea and malaria • Knowledge of newborn feeding and nutrition
Newborn characteristics	• Birth weight of baby • Breastfeeding status
Maternal newborn care practices	• Breastfeeding practices • Newborn hygiene and care practices including newborn bath/cleaning, handwashing, regular washing of newborn's clothes, bedsheet, usage of mosquito bite protection methods • Breast cleanliness • Personal hygiene of mother
Environmental sanitation practices	• Handwashing by all newborn handlers • Usage of separate footwear inside house • Feet cleanliness when sharing bed with baby • Cleanliness of bedsheet and floor disinfection
Tobacco and alcohol use	• Smoking history, type, frequency, quantity and duration in mother or father • Exposure to tobacco smoke • Chewing tobacco use in mother or father • Alcohol use history, type, frequency and quantity in mother or father • Other substance abuse in mother or father
Weight and weight gain	• Weight and weight gain during pregnancy
Cooking and drinking water	• Type of cooking fuel • Exposure to smoke from cooking fuel • Source of water used for cooking and drinking • Drinking water storage
Physical examination of neonate	
Anthropometry	Body length Weight Head circumference
Swab test	
Breast swab of mother	• A swab was collected from the nipple and another from the areola and cultured to identify the organisms presenting risk of infection to the breastfeeding neonates.
Bed swab	• One swab was collected from the bed or floor where the neonate is rested while sleeping.
Mouth swab of neonate	• A swab was collected from around the neonates' mouth and nostrils.
Hand swab of the caretaker	• Hand swabs were collected from the immediate caretaker of the baby including the mother and grandmother.

Follow-up visit on day 3 and 7 of life	
Newborn characteristics	• Weight of the baby • Breastfeeding status
Maternal newborn care practices	• Breastfeeding practices • Newborn hygiene and care practices including newborn bath/cleaning, handwashing, regular washing of newborn's clothes, bedsheet, usage of mosquito bite protection methods • Breast cleanliness • Personal hygiene of mother
Environmental sanitation practices	Handwashing by all newborn handlers Personal hygiene of the newborn's caretaker Usage of separate footwear inside house Feet cleanliness when sharing bed with baby Cleanliness of bedsheet and floor disinfection
Dietary questionnaire	A mix of closed and open-ended questions were used to capture questions on meal pattern, frequency of consumption, food intake changes after delivery
Health events questionnaire	Questions on health events for mother and newborn since the last visit.
Physical examination of neonate	
Anthropometry	Body length Weight Head circumference
Swab test	
Swabs	Six swabs as described above in table namely breast swabs (2), mouth swab of neonate (1), bed/surface swab (1), hand swabs of mother and caretaker (2) were collected.
Components of outcomes visit on day 28 of life	
Measurement	
Questionnaires	• Survival status
	• Health events between 7-27 days and treatment sought
	• General Health
Anthropometry of newborn	• Length
	• Weight
Intervention	• Advice on infant and maternal health and nutrition
	• Education about danger signs

[Table/Fig-2]: Components of study baseline and follow-up assessment.

Laboratory Procedures and Identification of Organisms

Six swabs from each participant, as described in [Table/Fig-2], were inoculated on blood agar and Mac Conkey agar aseptically. The plates were kept in incubator at 37°C for 18-24 hours. The growths from blood agar and Mac Conkey agar were assessed from initial observation after incubation for nature of isolated colonies to decide whether additional procedures were required or not. Relevant parameters included type of colony, gram reaction, morphology of bacteria studied, as outlined below.

Type of Colonies

On blood agar, colonies were interpreted as haemolytic or non-haemolytic, grey/white/yellow coloured and opaque/transparent. On Mac Conkey agar, colonies were interpreted as lactose fermenting or non-fermenting (based on presence of pink colour or colourless respectively).

Gram Reaction

Smears prepared from isolated colonies were subjected to gram staining. The stained smears were observed under microscope and interpreted as gram positive or negative, as well as morphology as cocci and bacilli.

Identification of Organisms

If the organism was gram positive cocci, catalase test was done. A positive catalase test indicated staphylococcus. Coagulase test was done to differentiate *staphylococcus aureus* from Coagulase Negative Staphylococcus (CONS).

If the catalase test was negative, organism was identified as streptococcus. Bile Esculin hydrolysis test was done to identify enterococcus, which shows positive test.

If the organism was gram negative bacilli, few relevant biochemical reactions including indole test (I), Citrate test (C), Urease test (U) and Triple Sugar Iron test (TSI) were done. The combinations of reactions used for organism identification are shown in [Table/Fig-3]. Further identification was done based on standard guidelines [18].

Training and Quality Control

All the study investigators and laboratory technicians required training before the start of the study. Two investigators were trained for one week in the Department of Community Medicine prior to study commencement by certified faculty on study protocol, administering the questionnaire, practice with data form completion, various measurements used in the study and collection and transportation of swabs. The laboratory technician was trained in protocol pertaining to processing of swabs, preparation of required culture media, and incubation of the inoculated plates in the microbiology department for one day. The entire process was supervised by a microbiologist. The growths on culture media were tested, identified and reported by a certified microbiologist.

Data quality was assessed through weekly audit of the data forms. All equipment was calibrated frequently to ensure proper functioning. Control plates were used for identifying commensals. A total of 10% of the culture results were cross verified by a senior microbiologist for ensuring data quality.

Test findings	Organism
TSI:A/A with little gas + I: positive + C: negative + U : negative	*Escherichia coli (E. coli)*
TSI:A/A with plenty of gas + I: negative + C: positive + U : positive	*Klebsiella*
TSI:A/A + I: positive + C: positive + U : negative	*Citrobacter*
TSI: K/no change + I: negative + C: positive + U: negative	Further identification based on oxidase test and motility: Oxidase positive, motile: *Pseudomonas* Oxidase negative, nonmotile: *Acinetobacter* Oxidase negative, motile: *Stentrophomonas* Oxidase positive, nonmotile: nonFermenter

[Table/Fig-3]: Test reactions and identification of gram negative organism.

Surveillance for Neonatal Sepsis

Community-level surveillance of neonatal illness

Routine household visits were scheduled on postnatal days 3, 7 and 28. At each postnatal visit, investigator assessed the newborns, identified the presence of illness, and made referrals to Mediciti Hospital according to a clinical algorithm designed in consultation with paediatric and community health experts. This algorithm was derived from Integrated Management of Neonatal and Childhood Illnesses (IMNCI) guidelines, protocol for neonatal sepsis at All India Institute of Medical Sciences, New Delhi and local clinical presentation of neonates with sepsis [19,20].

'Neonatal sepsis' was considered to be present if neonate aged between 7-28 days had signs of septicemia, meningitis, pneumonia, arthritis, osteomyelitis or urinary tract infection. Presence of at least one or more of the following symptoms or signs including stopped suckling, fever or cold to touch, unresponsive or unconscious or lethargic, bulging fontanelle, convulsions, absent reflexes or hypotonia, brady/tachycardia, vomiting, hyperbilirubinemia, redness or drainage from umbilical stump, skin bumps containing pus or blisters or single large area of pus with swelling, bleeding or petechiae, chest in drawing, fast breathing or local term for pneumonia implied 'possible neonatal sepsis'. Illnesses requiring referral included Very Severe Disease (VSD), possible VSD (PVSD), and diarrhea with blood or severe dehydration.

For referred neonates, Community Health Workers (CHV) facilitated transportation, and all care at the hospital was offered free of cost. Investigator assigned the aetiologic cause for sepsis, based on blood culture reports. After the first 28 days of life, investigator contacted participants/CHVs to record survival status of neonates.

Hospital-level Surveillance of Neonatal Infections

Infants were visited on postnatal day 1 in the hospital, coinciding with enrolment visit. The investigator recorded their health status. Risk factors for infection were listed using a pre-designed checklist. If a baby developed infection, he was classified as early onset neonatal sepsis, and excluded from further follow-up visits. As a routine, blood cultures were recommended by physicians for all neonates with: 1) clinical suspicion of possible serious infection; or 2) antibiotic treatment recommendation by the physician. Blood cultures were performed by the lysis direct plating method using 2 ml of blood using standard techniques, followed by anti-biograms [21]. Primary culture reports of growth or no growth were provided to the clinical care team within 18-24 hour. Results were categorised as sensitive, intermediate, and resistant on the basis of standard methods.

STATISTICAL ANALYSIS

All data were analysed using the SPSS 21 software (SPSS Inc., Chicago, IL, USA). For this paper, incidence rate of probable sepsis was calculated and reported as rate with 95% confidence intervals. The key background variables were reported as proportions. Frequencies were calculated, and mean and standard deviation was reported for normally distributed continuous variables. The numbers of organisms isolated from different surface swabs were also reported.

RESULTS

Socio-demographic and Other Characteristics of Included Newborns

One hundred participants were enrolled and analysed. Total 97% of the respondents were mothers. The socio-demographic characteristics of the mothers are shown in [Table/Fig-4]. The mean age of mothers at the time of current delivery was 21.52 (±1.55) years. Total 96% were literate and 54% were employed in income generating occupations.

Selected maternal and child health characteristics are listed in [Table/Fig-5]. Total 64% of the mothers were primigravida and 94% received complete antenatal care.

Characteristics	Mean±SD (N=100)
Age of mother, Mean (±SD), years	21.52±1.55
Years of schooling, Mean (±SD), years	7.34±3.56
Currently working (%)	54
Lowest income quartile (<18625 INR per month) (%)	27
Family size, Median (IQR)	5 (1-6)

[Table/Fig-4]: Socio-demographic characteristics of the cohort at baseline.

Characteristics	Percentage (N=100)
Mother primigravida	64
Preterm delivery	16
Low birth weight baby (<2500 grams)	31
Delayed initiation* of breastfeeding	94
Mother washed hands before breastfeeding	
Day 1	59
Day 3	73
Caretaker NOT washed hands before handling baby**	98
Babies exposed to pathogenic organisms in hospital	52
Babies exposed to pathogenic organisms in community#	34

[Table/Fig-5]: Maternal practices and child exposure measures among study participants on follow-up.
*Delayed initiation of breastfeeding was considered if breastfeeding was not initiated within one hour after birth; **Caretaker implies those members of the family involved in immediate care of the newborn, including grandmother, father, or aunts; #Total number of swabs with growth of pathogenic organisms for all sites included together, collected from their households

Maternal knowledge and practices were assessed at the time of recruitment and also during the third and seventh follow-up day in the community. The key knowledge parameters that significantly improved on the third follow-up compared with recruitment were knowledge of danger signs of pregnancy (22% during recruitment to 61% during first follow-up), benefits of institutional delivery (54% to 77%) and knowledge about danger signs in neonates (31% to 75%) and prevention of hypothermia, diarrhea, respiratory infection and malaria in neonates (46% to 81%). The practices of mothers related to feeding of colostrum (46% having knowledge at baseline to 67% practicing in first follow-up), and handwashing practices before feeding and handling the newborn improved on third follow-up (59% during first follow-up to 73% in second follow-up), while the others remained unchanged on second follow-up.

Outcomes of Study Participants based on Community Level Surveillance of Neonatal Illnesses

The outcomes of enrolled neonates during the study period and after 27 days of age are shown in [Table/Fig-6]. No neonatal mortality was recorded among the enrolled neonates. Severe jaundice was not included since the cause, whether direct or indirect hyperbilirubinemia could not be ascertained.

Outcomes	Total n (%) (N=42)
Mortality	0(0)
Possible neonatal sepsis	32 (76)
Fever*	13 (31)
Diarrhea*	13 (31)
Respiratory infection*	6 (14)
Meningitis*	0(0)
Severe jaundice**	10 (23)
Minor sickness not requiring hospitalisation#	5 (12)

[Table/Fig-6]: Outcomes among neonates enrolled in study.
*Fever, diarrhea, respiratory infections and meningitis were considered together as possible neonatal sepsis, according to the study algorithm. Numbers shown for these four conditions reflect the total number with possible neonatal sepsis; **Severe jaundice was considered wherever a written diagnosis by the treating doctor from the health facility where the baby was taken for treatment; # Minor sickness included all condition where the newborn was reported to be sick by the mother, but got better with home based treatment, or did not require to be admitted to a hospital for treatment. Conditions included fever, vomiting, rash

Forty-two neonates were hospitalised after 7th day of life.The CANS (culture negative) rate was 3.23 per 1000 live births (95% CI: 0.00-4.28; p=0.01). The mean duration of hospitalisation was 1.29 (+0.55) days. No complications were reported during hospital stay.

Outcomes of Study Participants based on Hospital based Surveillance of Neonatal Infections

Only one neonate (three-day-old) was admitted to Mediciti hospital paeditric ICU during the entire study period whose blood culture was negative for sepsis. Therefore, it was inferred that there were no culture positive neonatal bacteremia.

Organisms Isolated

The organisms isolated from different surface swabs in hospital and during follow-up visits in community are shown by site of swab collection in [Table/Fig-7]. Overall, the neonates showed 60.4% prevalence of pathogenic organisms from various surface swabs, of which 83% were isolated from institution and 54.5% from households. The most common organisms isolated from different surfaces in hospital were coagulase negative Staphylococci and Klebsiella, while *E.coli*, Citrobacter, Acinetobacter, Pseudomonas and non-fermenters were additionally isolated from household surfaces. *Staphylococcus aureus* was highly prevalent in hospital compared to home [Table/Fig-7].

Caretaker's hands showed the highest number of organisms, both in hospital (26%) and in community (12%). The other sites harboring organisms in households were mothers' areola (n=8), neonates' mouth (n=7), bed or floor where neonate rested (n=7), mothers' hands (n=5) and mothers' nipples (n=3) in descending order. Overall, Klebsiella was the commonest organism isolated at both hospital and home while Enterococcus was not isolated in any swab from home.

Organism	Neonate's mouth		Mother's nipple		Mother's areola		Mother's hand		Caretaker's hand		Bed/floor	
	Hospital# (n=100)	Home## (n=115)	Hospital# (n=100)	Home## (n=115)	Hospital# (n=100)	Home## (n=115)	Hospital# (n=100)	Home## (n=115)	Hospital# (n=100)	Home## (n=115)	Hospital# (n=100)	Home## (n=115)
Klebsiella	5	1	0	2	1	2	0	2	18	5	4	1
E. coli	0	0	0	0	1	1	1	1	4	4	2	1
Non-fermenters	0	0	0	0	0	0	0	0	1	1	0	0
Enterococci	1	0	1	0	0	0	0	0	0	0	0	0
Pseudomonas	1	0	0	0	0	0	0	0	1	0	0	1
Staphylococcus aureus	4	0	0	0	0	1	2	0	1	0	4	0
Coagulase negative *staphylococcus*	9	6	6	1	7	3	6	2	1	2	1	3
Citrobacter	0	0	0	0	0	0	0	0	0	0	0	1
Acinetobacter	0	0	0	0	0	1	0	0	0	0	1	0
TOTAL	20	7	7	3	9	8	9	5	26	12	12	7

[Table/Fig-7]: Total organisms isolated by site.
#Samples from the hospital were collected on day 1 following delivery for all 100 neonates in the study. ##Home samples were collected on day 3 and 7 for the available participants on those days, therefore the total number of home samples n=115 includes samples collected on both days

DISCUSSION

The incidence of community acquired sepsis was low in the present study, diagnosed according to a clinical algorithm. The morbidities considered for diagnosis were of severe magnitude requiring hospitalisation of at least one day. We did not find any case of culture confirmed sepsis, since none of the neonates were brought back to the hospital. We reported culture negative community acquired sepsis rate of 3.23/1000 live births, which was much lower than 6.7/1000 live births reported from Odisha in Eastern India (culture positive), or 2% from North India (culture positive) or 30/1000 live birth from data from the National Neonatal-Perinatal Database (2002-2003) [22-24]. A recent multicentric study including two centers from India reported the rates of culture positive bacterial infection among neonates as 10.61/1000 live births (95% CI: 7.44-14.89) from Vellore in South India and 23.46/1000 live births (95% CI: 18.52-29.27) from Odisha [25]. Amongst other developing countries, population-based incidence rate of 2.9/1000 live births for culture-confirmed community-acquired neonatal infection was reported from Mirzapur, Bangladesh [26]. Lower early-onset neonatal sepsis incidence rate of 1.64/1000 live births was reported in the United States in 1998-2000 while 3.2/1000 live births incidence was reported from Southern Israel [27,28].

The present study showed a high burden of pathogenic organisms isolated from various sites in mothers' and caretakers' body as well as the neonates' body and surface of resting. The proportion of organisms was higher in hospital compared with home on the seventh day. The pattern of organisms isolated varied in their proportions in hospital and home suggesting that organisms existing in community were different than hospitals. While CONS and Klebsiella was the predominant organisms isolated from hospital, Citrobacter was additionally isolated from home. Pattern of isolation of organisms in other population and facility based studies from developing and developed countries have similarly showed Staphylococcus aureus as the most prevalent [29-31]. Studies from rural Philippines and Israel found predominance of gram negative pathogens such as *Klebsiella pneumoniae* [28,32].

Historically, a study from Pondicherry in India done in 1986 presented their results on organisms isolated from 245 newborns having neonatal conjunctivitis. The most common organisms were Staphylococcus, Pseudomonas and Klebsiella [33]. A similar pattern of isolated organisms among neonatal sepsis patients still persists. Staphylococcus was the commonest organism isolated in studies from Northern India [34,35]. More recent studies from Southeast Asia including Pakistan, Bangladesh and South India have mostly found Gram negative organisms such as Klebsiella, Enterobacter, *E.coli*, Pseudomonas and *Staphylococcus aureus* [26,29,34-37]. DeNIS, a large study from Delhi reported Acinetobacter (22%), Klebsiella (17%) and *E. coli* (14%) isolated from neonates with EoNS and LoNS among hospital deliveries [38].

Overall, the organisms isolated among hospital delivered neonates include Klebsiella, *Staphylococcus aureus* and *E. coli*, as well as GBS wherever reports are available, in developing countries. For home deliveries, most studies have not been able to isolate, in-spite of a strong suspicion for GBS to be the causative organism for CANS [39].

When stratified according to site, the surface where the baby was rested/slept bore the highest number of pathogens both in hospital and home. This is possible since it is a cultural practice

to place the baby together with mother. It is also common for rural people to walk barefoot. Since mothers and other caretakers did not wash their feet before climbing on the bed, the organisms harbored in feet contaminated the surface. This was nearly significant upon univariate analysis (not shown here). It may therefore, be proposed that apart from handwashing, washing feet before going to bed can also prevent exposure to multiple organisms. A high proportion of organisms were isolated from caretakers' hands also implying the importance of proper handwashing by all caretakers or visitors who handle the baby in hospital or at home. This has been shown to be a significant risk factor in other studies also [4,40].

It is noteworthy that in few studies importance of surface cultures from the neonates body have shown its limited utility in predicting pathogens responsible for sepsis. A study from Dhaka found the sensitivity and specificity of skin cultures as only 16% and 38% among hospitalised preterm neonates [41]. Another similar study used skin cultures from different neonatal sites and reported the optimum sensitivity and specificity to be 56% and 82% respectively with very low PPV of 7.5%, thereby concluding on the limited value of surface cultures in aetiology of sepsis causing organisms [42].

Overall, the pattern of isolation of organisms from confirmed sepsis cases in previous studies did not seem to differ much from the exposures to neonates in this study. The recent paper on neonatal sepsis in South Asia reiterates findings from this study, while supporting the fact for a possibility of higher number of culture negative sepsis [43]. Exposure to surface pathogens may lead to CANS, and therefore these exposures must be prevented to the largest possible extent. This study has reported different sites in the immediate environment of the newborn from where the exposures can happen. Further studies may be undertaken to explore the home environment in total that may help to establish a stronger link between these exposures and outcomes.

This study was novel and important in several ways, most notably in its characterisation of organisms associated with neonatal sepsis in hospital and at home of rural newborns. This is the first study in India, to objectively demonstrate the presence of pathological agents in immediate environment of the newborns; and to report the personal hygiene and environmental sanitation practices of mothers and caretakers of newborns potentially exposing them to the risk of infections.

This knowledge presents with very unique opportunity to identify the faulty behaviours in households and rectify them for lowering the prevalence of community acquired sepsis.

The development and use of a clinical algorithm for community surveillance was attempted in this study in the absence of laboratory confirmed sepsis and related mortality, that has rarely been used in the past, lending present findings to international comparisons. Few studies have however, shown the limited implications of surface pathogens in causing sepsis, but the associations, although weak, have not been ruled out altogether [41,42]. This study therefore, behold importance to explore these relationships in greater detail. Although knowledge and practices of mothers pertaining to child care have been frequently studied in the past, very few have attempted to study its relationship with risk of neonatal sepsis. The study was also meant to see the feasibility of collecting samples from various sites and testing them in a central laboratory.

Limitation(s)

The recruitment reflected the study goal of recruiting committed individuals who agreed to be followed-up for 28 days. Therefore, the generalisability of the study was limited in favor of internal validity. Another limitation of this study is its setting, where the organisms associated with home deliveries that are more representative of community acquired sepsis could not be explored. Secondly, the number of preterm and premature births was also insufficient which limited the study to bring out the associations between prematurity and risk of future sepsis. Finally, the parents of sick newborns could not be motivated to return to hospital for a blood culture, so that the actual case definition could be ascertained.

An element of recall bias in reporting of diseases suffered by newborn since the previous visit on 7th day could not be ruled out on follow-up visit on the 28th day. The likelihood of the incidence rate to be an underestimation cannot be ruled out. Since, this study did not have scheduled visits in its design between 8th and 27th day of life, whereas almost all hospitalisations were reported in that duration, the probability of recall bias for reporting of sickness episodes remains. A routine follow-up between 7th and 28th day of life may be important in understanding of the incubation periods of different pathogens that the newborns are exposed to. It can further aid to increase compliance for timely referrals in the event of sickness.

CONCLUSION(S)

Newborns delivered at institution were exposed to various organisms at hospital and home. Further longitudinal studies may be undertaken to study the attack rates of the isolated organisms for causing sepsis. Collected samples from all sites showed a high prevalence (60.4%) of pathogenic organisms from various surface swabs both in institution (83%) and household (54.5%). The most common organisms isolated from different surfaces in hospital as well as home were, coagulase negative *Staphylococci, Klebsiella* and *E.coli*. *Staphylococcus aureus* was highly prevalent in hospital compared with home. Exposures of pathogenic organisms to neonates were high in hospitals than at homes and more via poor hand hygiene.

Thus, it is important to maintain a strictly aseptic environment in the hospital to reduce the risk of nosocomial infections and to extend the stay of newborns till first week of life to reduce the risk for community acquired sepsis resulting from exposure to organisms at home during periods of comparatively weaker immunity. Home environment must be revisited and family members must be strongly advised to maintain clean environment at home.

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Author contribution: EG and VK conceptualised and designed the study, defined the intellectual content, did literature search and prepared and edited the manuscript. VK and AP acquired the data and conducted the clinical study. KM and SK conducted the laboratory procedures and participated in writing the first draft. PKS did statistical analysis and edited the manuscript. All authors reviewed the manuscript and approved the final version. Both EG and VK have same contribution and equal credit should be given for the same.

REFERENCES

[1] World Health Organisation. Essential New-born Care: Report of a Technical Working Group, 1994. WHO/FRH/MSM/96.13, Geneva: World Health Organisation; 1996.
[2] Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, et al. Global, regional, and national causes of child mortality in 2008:A systematic analysis. Lancet. 2010;375:1969-87.
[3] Ministry of Health and Family Welfare. Family welfare statistics in India. New Delhi: Government of India, Statistics Division; 2011.
[4] Bang AT, Bang RA, Baitule S, Deshmukh M, Reddy MH. Burden of morbidities and the unmet need for health care in rural neonates-A prospective observational study in Gadchiroli, India. Indian Pediatr. 2001;38:952-65.
[5] Singh M, Deorari AK, Khajuria RC, Paul VK. A four year study on neonatal morbidity in a New Delhi hospital. Indian J Med Res. 1991;94(B):186-92.
[6] Bhakoo ON, Narang A, Kulkarni KN, Patil AS, Banerjee CK, Walia BNS. Neonatal morbidity and mortality in hospital born babies. Indian Pediatr. 1975;12:443-50.
[7] Jajoo M, Kapoor K, Garg LK, Manchanda V, Mittal SK. To study the incidence and risk factors of early onsetneonatal sepsis in an out born neonatal intensive care unit of India. J Clin Neonatol. 2015;4:91-95.
[8] Thaver D, Zaidi AK. Burden of neonatal infections in developing countries: A review of evidence from community-based studies. Pediatr Infect Dis J. 2009;28:S3-9.
[9] Bang AT, Bang RA, Tale O, Sontakke P, Solanki J, Wargantiwar R, et al. Reduction in pneumonia mortality and total childhood mortality by means of community-based intervention trial in Gadchiroli, India. Lancet. 1990;336(8709):201-06. doi: 10.1016/0140-6736(90)91733-q. PMID: 1973770.
[10] Bhardwaj N, Hasan SB. High perinatal and neonatal mortality in rural India. J R Soc Health. 1993;113(2):60-63. doi: 10.1177/146642409311300203. PMID: 8478893.
[11] Bang AT, Bang RA, Baitule SB, Reddy MH, Deshmukh MD. Effect ofhome-based neonatal care and management of sepsis on neonatal mortality: Field trial in rural India. Lancet. 1999;354(9194):1955-61.doi:10.1016/S0140-6736(99)03046-9. PMID: 10622298.
[12] Phukan RK, Mahanta J. A study of neonatal deaths in the teagardens of Dibrugarh district of upper Assam. J Indian Med Assoc. 1998;96(11):333-34, 337. PMID: 10218318.
[13] Singh M, Narang A, Bhakoo ON. Predictive perinatal score in thediagnosis of neonatal sepsis. J Trop Pediatr. 1994;40:365-68.
[14] Waters D, Jawad I, Ahmad A, Lukšić I, Nair H, Zgaga L, et al. Aetiology of community-acquired neonatal sepsis in low- and middle-income countries. Journal of Global Health. 2011;1(2):154-70.
[15] Bangi VA, Devi SS. Neonatal sepsis: A risk approach. J NTR UnivHealth Sci. 2014;3:254-58.
[16] Ganatra B, Hirve S. Male bias in health care utilisation for underfives in a rural community in western India. Bulletin of the World Health Organisation. 1994;72(1):101-04.
[17] Kusneniwar GN, Whelan M, Betha K, Robertson J, Reddy PR, Balasubramanian K, et al. Cohort Profile: The Longitudinal Indian Family Health (LIFE) Pilot Study, Telangana, India. Int J Epidemiol. 2017;46(3):788-89.
[18] Procop GW, Church DL, Hall GS, Janda WM, Koneman EW, Schreckenberger PC, et al. Koneman's colour atlas and textbook of diagnostic microbiology, 7th edition. Philadelphia, USA: Lippincott Williams and Wilkins; 2016.
[19] WHO. Students' Handbook for Integrated Management ofNeonatal and Childhood Illness. Ministry of Health and Family Welfare, GoI, 2003. https://www.jknhm.com/zip/IMNCI%20%20Students%27%20Handbook.pdf [Accessed December 20, 2016].
[20] Sankar MJ, Agarwal R, Deorari AK, Paul VK. Sepsis in the Newborn. AIIMS- NICU protocols 2008. https://www.newbornwhocc.org/pdf/sepsis_innewborn.pdf [Accessed December 20, 2016.]
[21] Cheesbrough M. Medical laboratory manual for tropical countries. London: Tropical Health Technology and Butterworth-Heinemann; 2000.
[22] Panigrahi P, Chandel DS, Hansen NI, Sharma N, Kandefer S, Parida S, et al. Neonatal sepsis in rural India: timing, microbiology and antibiotic resistance in a population-based prospective study in the community setting. J Perinatol. 2017;37(8):911-21.
[23] Baqui AH, Darmstadt GL, Williams EK, Kumar V, Kiran TU, Panwar D, et al. Rates, timings and causes of neonatal deaths in rural India. Bulletin of WHO. 2006;84:706.
[24] Report of the National Neonatal Perinatal Database (National Neonatology Forum); 2002-03. http://www.newbornwhocc.org/pdf/nnpd_report_2002-03.PDF. [Accessed December 24, 2018.]
[25] Saha SK, Schrag SJ, El Arifeen S, Mullany LC, Islam MS, Shang N, et al. Causes and incidence of community-acquired serious infections among young children in south Asia (ANISA): an observational cohort study. Lancet. 2018;392:145-59. doi:10.1016/S0140-6736(18)31127-9.
[26] Darmstadt GL, Saha SK, Choi Y, El Arifeen S, Ahmed NU, Bari S, et al. Population-based incidence and etiology of community-acquired neonatal bacteremia in Mirzapur, Bangladesh: An observational study. J Infect Dis. 2009;200(6):906-15. doi:10.1086/605473.

[27] Hyde TB, Hilger TM, Reingold A, Farley MM, O'Brien KL, Schuchat A. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. Pediatrics. 2002;110:690-95.

[28] Greenberg D, Shinwell ES, Yagupsky P, Greenberg S, Leibovitz E, Mazor M, et al. A prospective study of neonatal sepsis and meningitis in southern Israel. Pediatr Infect Dis J. 1997;16:768-73.

[29] Baqui AH, Rahman M, Zaman K, El Arifeen S, Chowdhury HR, Begum N, et al. A population-based study of hospital admission incidence rate and bacterial aetiology of acute lower respiratory infections in children aged less than five years in Bangladesh. J Health Popul Nutr. 2007;25:179-88.

[30] WHO Young Infants Clinical Signs Study Group. Bacterial etiology of serious infections in young infants in developing countries: results of a multicenter study. Pediatr Infect Dis J. 1999;18(Suppl 10):S17–22.

[31] Gatchalian SR, Quiambao BP, Morelos AM, Abraham L, Gepanayao CP, Sombrero LT. Bacterial and viral etiology of serious infections in very young Filipino infants. Pediatr Infect Dis J 1999; 18: S50–S55.

[32] Quiambao BP, Simoes EA, Ladesma EA, Gozum LS, Lupisan SP, Sombrero LT, et al. Serious community-acquired neonatal infections in rural Southeast Asia (Bohol Island, Philippines). J Perinatol. 2007;27:112-19.

[33] Pandey KK, Bhat BV, Kanungo R, Srinivasan S, Rao RS. Clinico-bacteriological study of neonatal conjunctivitis. Indian J Pediatr. 1990;57(4):527-31.

[34] Marwah P, Chawla D, Chander J, Guglani V, Marwah A. Bacteriological profile of neonatal sepsis in a tertiary-care hospital of Northern India. Indian Pediatr 2015;52:158-59.

[35] Pathak S, Agarwal D, Singh P, Pathak M, Narayan S. Late-onset neonatal sepsis: Overview of risk factors and bacterial etiology in a tertiary care hospital in North India. J Mahatma Gandhi Inst Med Sci. 2018;23:69-72.

[36] Bhutta ZA, Yusuf K. Neonatal Sepsis in Karachi: factors determining outcome and mortality. J Trop Pediatr. 1997;43:65-70.

[37] Aurangzeb B, Hameed A. Neonatal sepsis in hospital-born babies: bacterial isolates and antibiotic susceptibility patterns. J Coll Physicians Surg Pak. 2003;13(11):629-32.

[38] Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: A cohort study. Lancet Glob Health. 2016;4:e752-60.

[39] Kuruvilla KA, Thomas N, Jesudasan MV, Jana AK. Neonatal group B Streptococcal bacteraemia in India: Ten years'experience. Acta Paediatr. 1999;88:1031-32.

[40] Robillard PY, Narberth P, Hulsey TC, Sergent MP, Perianin J, Janky E. Neonatal bacterial septicemia in a tropical area. Four-year experience in Guadeloupe (French West Indies). Acta Pediatr. 1993;82:687-89.

[41] Choi Y, Saha S, Ahmed N, Law P, Azad Chowdhury MAK, Islam M, et al. Routine skin cultures in predicting sepsis pathogens among hospitalised preterm neonates in Bangladesh. Neonatology. 2008;94:123-31. 10.1159/000119722.

[42] Evans ME, Schaffner W, Federspiel CF, Cotton RB, McKee KT, Stratton CW. Sensitivity, specificity, and predictive value of body surface cultures in a neonatal intensive care unit. JAMA. 1988;259(2):248-52. doi:10.1001/jama.1988.03720020050036

[43] Chaurasia S, Sivanandan S, Agarwal R, Ellis S, Sharland M, Sankar MJ. Neonatal sepsis in South Asia: huge burden and spiralling antimicrobial resistance. BMJ. 2019;364:k5314.

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