Original Article

Blood Culture Confirmed Bacterial Sepsis in Neonates in a North Indian Tertiary Care Center: Changes over the Last Decade

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SUMMARY: The spectrum of organisms causing sepsis is different in developing countries. Data on the recent trends of organisms causing sepsis are limited. This study was conducted in a tertiary care neonatal unit in Northern India. All inborn babies with blood-culture-positive sepsis from 1995 to 2006 were divided into two epochs, viz. 1995 to 1998 (epoch I) and 2001 to 2006 (epoch II). Organisms were grouped into early (<72 h) and late onset (≥72 h) sepsis groups. The overall incidence of sepsis, the incidence of sepsis stratified by weight groups, the organism profile on different days of life, sepsis-related mortality and pathogen-specific case fatality rate were calculated and compared between the two epochs. Out of 34,362 live births during the study period, organisms were isolated in 1,491 neonates. Out of these, 89% had bacterial sepsis. The incidence of neonatal bacterial sepsis increased from epoch I to epoch II (35.8/1,000 versus 40.1/1,000 live births, P < 0.05). The incidence of early onset sepsis (EOS) did not change between the epochs, but the incidence of late onset sepsis (LOS) increased from 12 to 16.5 per 1,000 live births (P < 0.001). The incidence of bacterial sepsis decreased significantly in the 1,000- to 1,999-g birth weight groups. Klebsiella pneumoniae and Enterobacter aerogenes decreased, whereas Staphylococcus aureus increased in incidence during epoch II. Non-fermenting Gram-negative bacilli emerged as a newly identified pathogen during epoch II. Sepsis-associated mortality decreased from 42 to 20%. The incidence of bacterial sepsis has decreased significantly in 1,000- to 1,999-g infants, with a significant reduction in sepsis-related mortality. New organisms have emerged in recent years. The organism profile in recent years has changed, with a significant overlap of organisms causing EOS and LOS.

INTRODUCTION

Neonatal infections are estimated to cause 1.6 million deaths every year globally, and 40% of all neonatal deaths occur in developing countries (1,2). The spectrum of organisms causing neonatal sepsis is quite different in developed countries in comparison with developing countries like India (3,4). Within developing countries, regional variation exists in the spectrum of organisms causing sepsis (5,6).

Significant changes have occurred in the care of the sick and of immature newborns over the last decade. The use of antenatal steroids has become more prevalent, and surfactant is used in almost all preterm babies with respiratory distress. These have increased the survival of such babies, but may also have influenced the incidence and the profile of organisms causing sepsis. However, data on the recent trends of organisms causing sepsis in developing countries are limited (7). Hence, we conducted this study to analyze the changes in the incidence of bacterial sepsis and its causative organisms over the last decade.

MATERIALS AND METHODS

This retrospective study was conducted in a tertiary care neonatal unit in Northern India. All babies born in the hospital from 1995 to 2006 formed the baseline population. Because...
species level by conventional biochemical and serological tests (8). The growth of an organism was defined as pathologic if the same organism was isolated from both the broths and contaminant, if either the growth of the organism was detected in only one of the two broths or a mixed growth was detected. In babies in whom coagulase-negative Staphylococcus aureus (CONS) was isolated in the first 3 days of life, a repeat blood culture was performed to confirm the infection. Proven sepsis was defined as the presence of clinical features of sepsis along with the isolation of an organism in the blood culture.

All organisms were classified based on the time-point at which the blood was collected for culture as follows: those causing early onset sepsis (EOS—less than and up to 72 h of life) and those causing late onset sepsis (LOS—greater than 72 h of life). The organisms were further classified based on their Gram’s stain status.

The baseline demographic data for all included neonates, the overall incidence of sepsis, the incidence of sepsis stratified by birth weight groups, sepsis-related mortality and pathogen-specific case fatality rate (CFR) were calculated and compared between the two epochs. Baseline statistical analysis was done by Student’s t test or the Mann-Whitney U test for continuous variables and the chi-square test or Fisher’s exact test for categorical variables. Change in the profile of organisms over the years was analyzed by the chi-square test for linear trends.

RESULTS

A total of 34,362 neonates were born during the study period (12,171 in epoch I and 22,191 in epoch II). Overall, nearly one-third of the babies were preterm while close to half were low birth weight (LBW). The total number of live births as well as the proportion of preterm, LBW, very low birth weight (VLBW) and extremely low birth weight (ELBW) babies increased significantly during epoch II. The baseline characteristics of the study population are given in Table 1. Blood culture was positive in 1,491 neonates, from whom 1,647 organisms were isolated. In 89% of these neonates (n = 1,328, 38.6 per 1,000 live births) the isolates were bacterial, and were labeled as proven bacterial sepsis. The remaining 163 (11%) were fungal infections of which more than 90% were Candida spp. In this paper, the data for the bacterial organisms is presented. The overall incidence of bacterial sepsis increased from epoch I to epoch II (35.8/1,000 live births versus 40.1/1,000 live births; P = 0.05). Overall, EOS constituted 62% of all cases of sepsis. There was no change in the incidence of EOS between the two epochs (24 versus 23.8 per 1,000 live births), but there was an increase in the incidence of LOS from 12 to 16.5 per 1,000 live births (P < 0.001) from epoch I to epoch II. LOS as a proportion of neonatal bacterial sepsis also increased from epoch I to epoch II (33 to 41%, P = 0.01).

The incidence of neonatal bacterial sepsis, stratified for birth-weight categories, was then compared between the two epochs (Table 2 and Figure 1). There was a statistically significant reduction in the incidence of bacterial sepsis in the 1,000- to 1,249-, 1,250- to 1,499- and 1,500- to 1,999-g birth weight groups between the two epochs. In babies with birth weights less than 1,000 g and those weighing 2,000-2,499 g, there was a trend towards an increase in the incidence of sepsis, but it did not reach statistical significance.

The relative distribution of various organisms during both

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<tbody>
<tr>
<td>Total live births</td>
<td>12,171</td>
<td>22,191</td>
<td>-</td>
</tr>
<tr>
<td>Preterm (&lt;37 weeks)</td>
<td>3,809 (31)</td>
<td>8,172 (37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ELBW (&lt;1,000 g)</td>
<td>157 (1)</td>
<td>559 (3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLBW (&lt;1,500 g)</td>
<td>847 (7)</td>
<td>2,591 (12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LBW (&lt;2,500 g)</td>
<td>4,757 (39)</td>
<td>10,297 (46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SGA (&lt;2SD)</td>
<td>486 (4)</td>
<td>1,155 (5)</td>
<td>0.2</td>
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ELBW, extreme low birth weight; VLBW, very low birth weight; LBW, low birth weight; SGA, small for gestational age.

<table>
<thead>
<tr>
<th>Birth weight group (g)</th>
<th>Live births (n = 12,171) (%)</th>
<th>Incidence of sepsis (n = 437)</th>
<th>Survival (n = 293) (%)</th>
<th>LOS (n = 144) (%)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>500-749</td>
<td>34 (0.3)</td>
<td>6 (18)</td>
<td>4 (12)</td>
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<tr>
<td>750-999</td>
<td>123 (1.0)</td>
<td>19 (15)</td>
<td>11 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,000-1,249</td>
<td>314 (2.5)</td>
<td>76 (24)</td>
<td>39 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,250-1,499</td>
<td>376 (3.1)</td>
<td>80 (21)</td>
<td>34 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,500-1,999</td>
<td>1,332 (11)</td>
<td>67 (5)</td>
<td>33 (2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,000-2,499</td>
<td>2,578 (21)</td>
<td>28 (1.1)</td>
<td>12 (0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2,500</td>
<td>7,414 (61)</td>
<td>17 (0.3)</td>
<td>11 (0.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P value for comparison of incidence of sepsis in different birth weight groups between the two epochs. EOS, early onset sepsis; LOS, late onset sepsis.

![Fig. 1. Incidence of sepsis in different weight groups.](image-url)
the study epochs is given in Table 3. *Klebsiella pneumoniae* was the most common organism responsible for EOS during epoch I (32%), whereas non-fermenting Gram-negative bacilli (NFGNB) emerged as the most common pathogen causing EOS during epoch II (28%). *Enterobacter aerogenes* was the most common organism causing LOS during epoch I (35%), whereas *S. aureus* (31%) became the dominant organism causing LOS during epoch II. NFGNB, *Burkholderia cepacia* complex (BCC) organisms and *Enterococcus faecalis* were identified only during epoch II (2001-2006) and were conspicuously absent prior to 2001. NFGNB were isolated from both EOS and LOS, though nearly two-thirds (161/221 [73%]) of these were seen in cases of EOS. There was a significant reduction in the incidence of *E. aerogenes* and *K. pneumoniae* sepsis from epoch I to epoch II.

There was a significant increase in the proportion of sepsis due to *S. aureus* from epoch I to epoch II (81 [18.5%] versus 220 [25%], *P* = 0.01). This was predominantly caused by an increase in the proportion of LOS due to *S. aureus* from epoch I to epoch II (18 versus 31%, *P* = 0.003). On the other hand, there was no change in the proportion of EOS due to *S. aureus* between the two epochs (19 versus 21%, *P* = 0.5).

Sepsis-associated mortality decreased significantly from epoch I to epoch II (42% versus 25%, OR 0.36 [0.27 - 0.47], *P* < 0.001) (Table 4). Neonatal mortality rate (NMR) due to sepsis also decreased from epoch I to epoch II (20% versus 10%, *P* < 0.001). During epoch I, *K. pneumoniae* and *E. aerogenes* caused the highest rates of fatality with CFRs of 55 and 54%, respectively. During epoch II, the CFRs for almost all organisms decreased significantly, with the highest CFRs due to *Pseudomonas* (42%), *E. coli* (41%), and *A. anitratus* sepsis (41%).

**DISCUSSION**

In developing countries, rates of blood stream infections (BSI) have been reported to be 1.7 to 33 per 1,000 live births, with rates in Asia clustering around 15 per 1,000 live births (7). A report from 18 centers across India (National Neonatal Perinatal Database [NNPD]) for 2002-2003 reported an incidence of 8.5 per 1,000 live births for blood-culture-proven sepsis (9). Our study population is different from those in the previous studies in that it consisted of more high-risk neonates, with nearly 10% of the total live births being VLBW infants and 1.8% being ELBW infants. For true comparisons, a further description of population characteristics, preferably the severity of illness and intensity of therapeutic interventions, is required.

We observed an apparent increase in the overall incidence of neonatal sepsis from epoch I to epoch II primarily due to...
an increase in LOS. This increase occurred almost exclusively in babies with birth weight less than 1,000 g (26 versus 31%). In recent years, an increasing number of ELBW infants are surviving. Even amongst those who die, the age at death has increased. The resultant longer hospital stays and use of invasive devices and catheters makes these infants more vulnerable to nosocomial sepsis. However, in the same period, the incidence of sepsis decreased significantly in the 1,000- to 1,999-g weight groups. A similar change was observed by Philip in a regional neonatal intensive care unit from 1983 through 1992 in two epochs (10). He observed a significant increase in LOS, primarily nosocomial, in epoch II, with a proportionate decrease in very early onset sepsis (onset in less than 24 h) especially in VLBW infants. The reduction cannot be explained by a single factor. Multiple changes have occurred in recent years with increasing awareness of prevention of sepsis which could have contributed to the decrease. These changes include earlier and more aggressive enteral feeding and the resultant early discontinuation of vascular catheters, shorter duration of invasive ventilation because of surfactant use, better hand hygiene practices and better protocols for handling vascular lines, etc.

Gram-negative organisms were more common than Gram-positive organisms during both epochs and in both the EOS and LOS groups. However, we observed a significant increase in Gram-positive sepsis during epoch II. This increase was mainly attributed to an increase in *S. aureus* sepsis and the isolation of alpha-hemolytic streptococcus (*E. faecalis*). Although for many years Gram-negative bacteria were isolated from the majority of bacteremic patients with severe sepsis, the proportion of cases associated with Gram-positive bacteria has steadily increased over the last 2 decades, and now *S. aureus*, CONS, and *Enterococci* account for approximately 30 to 50% of the cases in most clinical series (11). *K. pneumoniae* was the major pathogen during epoch I and was responsible for 30% of blood-culture-positive sepsis cases, closely followed by *E. aerogenes* (26%). During epoch II, however, the incidence of both these organisms dropped significantly with the emergence of *S. aureus* (25%) and nonfermenters (23%) as the major pathogens. Data from both developed as well as developing countries have shown Gram-negative bacilli to be the major pathogens of neonatal sepsis (12,13). A review of 11,471 blood stream samples showed that Gram-negative rods were isolated from at least 60% of positive blood cultures in all of the developing regions of the world (7). Data from developing countries are more or less similar, with *K. pneumoniae* responsible for 16–28% of blood-culture-confirmed sepsis (7). Data from NNPD 2002-2003 from India has shown *K. pneumoniae* as the major pathogen in both intramural as well as extramural neonates (27-32%) (9). Group B streptococcus as a cause of neonatal sepsis has been reported very uncommonly from India (3,6,7).

*S. aureus* colonizes the skin, nasopharynx, and gastrointestinal tract and spreads via the hands of health care workers (14). Even though the reason for this drift in our study is not clearly understood, this increase in *S. aureus* LOS during epoch II could be related to the excessive load of VLBW babies without a concomitant expansion of facilities; this implies a need for better adherence to hand hygiene practices, cohorting and isolation, periodic surveillance, and decolonization of health care workers.

CONS were isolated less commonly in our study population. Latin America, Southeast Asia, and the Middle East have reported high rates of CONS infections, which might indicate a high rate of invasive device use (7). In our study, 55% of CONS were isolated in the first 48 h of life, with another 10% in the next 24 h. In 50% of these neonates, repeat blood cultures performed to rule out skin contamination grew CONS again, suggesting true infection. Stoll and Fanaroff analyzed the status of early onset CONS sepsis for the National Institute of Child Health and Human Development (NICHD), Neonatal Research Network (15). They identified 22 neonates who were blood-culture-positive for CONS on day 1 of life, and 11 out of these 22 were symptomatic and were subsequently labeled as true EOS with CONS.

NFGNB are the group of Gram-negative bacteria that do not ferment carbohydrates. This group comprises a long list of organisms including those of clinical importance like *Pseudomonas* spp., *Acinetobacter* spp., *BCC, Alcaligenes* spp., *Achromobacter* spp. etc. (16). It has always been a tedious task for a routine microbiological laboratory to identify NFGNB, and poor laboratory proficiency in their identification still prevails worldwide (17). With the assistance of the reference laboratory at Universiteit Gent, Belgium, conventional as well as molecular identification methods were used for the identification of certain NFGNB. Therefore, during the later part of epoch II (2005-2006), many non-fermenters were correctly identified and re-classified as BCC apart from the isolation of the previously described ones.

The BCC organisms represent a closely related group of NFGNB that survive and multiply in aqueous hospital environments including detergent solutions and intravenous fluids. Due to the high intrinsic resistance of the BCC to antibiotics and antimicrobial compounds, they can prove very difficult to treat (18). In our study population, 40% of neonates with BCC sepsis died. Of the NFGNB isolated in our study, 75% caused infection within the first 72 h of life (EOS). As of now, non-fermenters have not been reported to be isolated from the maternal genital tract (19,20). This strongly suggests that early onset hospital-acquired sepsis due to NFGNB should be an area of concern and future research.

To conclude, the incidence of neonatal bacterial sepsis has decreased significantly in the 1,000- to 1,999-g birth weight group of neonates in spite of their increased survival. The organism profile in recent years has changed significantly, with a reduction in *K. pneumoniae* sepsis, an increase in *S. aureus* sepsis and the emergence of new organisms such as NFGNB.

**ACKNOWLEDGMENTS**

We sincerely thank Dr P. Vandamme, Universiteit Gent, Belgium for assisting us in the molecular based identification of *Burkholderia cepacia* complex and related organisms.

**REFERENCES**


