Etiology of Bacteremia in Young Infants in Six Countries

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Background: Neonatal illness is a leading cause of death worldwide; sepsis is one of the main contributors. The etiologies of community-acquired neonatal bacteremia in developing countries have not been well characterized.

Methods: Infants <2 months of age brought with illness to selected health facilities in Bangladesh, Bolivia, Ghana, India, Pakistan and South Africa were evaluated, and blood cultures taken if they were considered ill enough to be admitted to hospital. Organisms were isolated using standard culture techniques.

Results: Eight thousand eight hundred and eighty-nine infants were recruited, including 3177 0–6 days of age and 5712 7–59 days of age; 10.7% (947/8889) had a blood culture performed. Of those requiring hospital management, 782 (54%) had blood cultures performed. Probable or definite pathogens were identified in 10.6% including 10.4% of newborns 0–6 days of age (44/424) and 10.9% of infants 7–59 days of age (39/358). *Staphylococcus aureus* was the most commonly isolated species (36/83, 43.4%) followed by various species of Gram-negative bacilli (39/83, 46.9%; *Acinetobacter* spp., *Escherichia coli* and *Klebsiella* spp. were the most common organisms). Resistance to second and third generation cephalosporins was present in more than half of isolates and 44% of the Gram-negative isolates were gentamicin-resistant. Mortality rates were similar in hospitalized infants with positive (5/71, 7.0%) and negative blood cultures (42/557, 7.5%).

Conclusions: This large study of young infants aged 0–59 days demonstrated a broad array of Gram-positive and Gram-negative pathogens responsible for community-acquired bacteremia and substantial levels of antimicrobial resistance. The role of *S. aureus* as a pathogen is unclear and merits further investigation.

Key Words: Neonatal sepsis, infant, neonate, bacteremia, *Staphylococcus aureus*

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Severe infections, specifically pneumonia, meningitis and sepsis, have been estimated to be responsible for 23% of the approximately 3 million global neonatal deaths that occur annually; an even larger proportion of neonatal and young infant fatalities due to serious bacterial infections may occur in high-burden community settings.[1,2] A review of 15 studies of neonatal sepsis in developing countries, performed in the late 1990s found that the most commonly encountered species in blood culture-positive cases were *Klebsiella* spp., *Escherichia coli*, Staphylococcus aureus and *Pseudomonas* spp.[3] In contrast to studies from industrialized countries, group B streptococcus (GBS) was rarely encountered. Since many of these studies from developing countries were hospital-based, it was likely that many of these infections were nosocomial and thus not reflective of community-acquired serious bacterial infections. A more recent review of 27 etiological studies found similar findings with *S. aureus* (14.9%), *E. coli* (12.2%) and *Klebsiella* spp. (11.6%) the most commonly encountered organisms in community-acquired neonatal sepsis.[4]

In order to further refine the Integrated Management of Childhood Illness algorithm for identifying sick young infants in need of referral, we performed a multi-country study that was designed to evaluate a broader range of noninfectious as well as infectious diseases in infants in the first 2 months of life. We attempted to enroll a substantial proportion of neonates in the first week of life in order to better characterize this highly vulnerable population.[5] We report here a summary of the etiologies and resistance patterns of serious bacterial infections from this multi-site study.

MATERIALS AND METHODS

Study Sites

Study sites included the Dhaka Shishu Hospital in Dhaka, Bangladesh; Hospital del Niño and Hospital Materno-Infantil in

La Paz, Bolivia; Komfo Anokye Teaching Hospital in Kumasi, Ghana; Postgraduate Institute for Medical Education and Research and General Hospital, Sector 16 in Chandigarh, India; All India Institute of Medical Sciences and Safdarjung Hospital in Delhi, India; 3 primary health clinics established for the study by the Department of Paediatrics and Child Health of the Aga Khan University in Karachi, Pakistan; and King Edward VIII Hospital in Durban, South Africa. Details of the study sites are available in site-specific reports.[6–10]

Patient Selection

Full details of the study design are described elsewhere, in conjunction with the study's primary analysis of the predictive value of clinical signs.[5] Briefly, children were included in the study if they were <60 days of age and were brought to the hospital or outpatient clinic for an acute illness. Infants were excluded if they were presenting for well-baby visits, did not reside in the defined study area (to ensure follow-up), had been previously enrolled or were being seen for a repeat episode of the same illness. Additional exclusion criteria included need for immediate cardiopulmonary resuscitation, hospitalization in the previous 2 weeks (except for delivery), referral from another health facility, an obvious lethal congenital malformation (eg, anencephaly) or caretaker unwillingness to provide informed consent. Thus, the study was designed to mimic a primary care setting as much as possible.

After providing informed consent, infants were referred to a trained primary health worker for initial evaluation of clinical signs using a standardized data collection form. After this assessment, the subject was referred to a study pediatrician who took a complete history, performed a physical examination and decided whether the infant required further hospital management including greater diagnostic evaluation or admission for inpatient treatment. The clinical course of hospitalized children was followed and the final outcome was documented. The care providers of infants who were not admitted to the hospital were advised to return in 48–72 hours for an evaluation in order to determine the outcome of the child's illness.

Microbiologic Methods

The protocol called for blood cultures to be performed on all admitted patients. In addition, the study protocol allowed for study physicians to obtain blood cultures on all infants with suspected sepsis, regardless of admission status as many families declined admission and preferred outpatient therapy. Samples were transported to the lab immediately and processed using standard methods (Appendix 1).[11] Antimicrobial susceptibility testing was done by the disc diffusion method using Mueller Hinton agar in accordance with Clinical and Laboratory Standards Institute performance standards for susceptibility testing.[12] Internal quality control was routinely performed at least weekly in all laboratories. All microbiology laboratories had some form of external quality control either through national or international regulatory agencies.

Blood cultures were considered positive if a definite pathogen was grown. The following isolates were considered to be contaminants: viridans streptococci, *Micrococcus* spp., *Bacillus* spp., diphtheroids, coagulase-negative staphylococci and *Candida* spp.[13]

Data Management and Analysis

Case record forms were checked for completion and correctness, and then double entered into an EpiData database (version 2.1, EpiData Association, Odense, Denmark) at each of the study sites. Data files were sent to the Data Coordination Centre at Murdoch Children's Research Institute (Melbourne, Australia), where further data cleaning and consistency checks were performed and the quality of data submitted from the individual sites was monitored.

Analysis was done using Stata version 11 software (StataCorp, College Station, TX). The frequency of organisms was described, as was the antimicrobial susceptibility, and the association of individual organism isolated with hospitalization and death was explored. Blood culture isolates classified as contaminants were treated as negative cultures for the purpose of analysis. The presence of specific pathogens was correlated with the clinical characteristics of the infection, which were categorized as either showing no signs of infection, focal, focal with systemic manifestations or systemic. Infections were classified as focal if they involved only the skin and soft tissues (eg, omphalitis, abscess, etc.) and lacked systemic signs of illness. By contrast, systemic infections included diagnoses of sepsis, meningitis and pneumonia. Antimicrobial therapy data were not routinely collected and therefore we are unable to describe treatments provided. Proportions were compared by χ^2.

Ethical Considerations

The study protocol was reviewed and approved by the institutional review boards or ethical committees of the study sites. The protocols for sites overseen by Boston University (Ghana, South Africa), through support from the United States Agency for International Development were also reviewed by the Boston University Medical Center institutional review board. For sites supported by Saving Newborn Lives (Bangladesh, Bolivia, Pakistan) through a grant from the Bill and Melinda Gates Foundation, the protocols were reviewed by the Johns Hopkins University institutional review board and determined to be exempt. The World Health Organization (WHO) Secretariat Committee on Research Involving Human Subjects reviewed and approved the protocols for WHO-supported sites (Delhi and Chandigarh, India).

RESULTS

Of the 8889 infants enrolled in the study, 1437 infants were classified as requiring urgent hospital management (Fig. 1). Of those requiring hospital management, 782 (54%) had blood cultures performed as per protocol; the analysis was limited to this group. Contaminants were identified in the blood cultures of 6.1% (48/782) including 5.4% (23/424) of newborns aged 0–6 days and 7.0% (25/358) of infants aged 7–59 days. Probable or definite pathogens were identified in 10.6% overall with similar proportions in newborns aged 0–6 days (10.4%, 44/424) and infants aged 7–59 days (10.9%, 39/358). Although all 782 were referred for hospitalization, 124 were not admitted due to shortage of beds, parental refusal and clinician decision. More than half of the latter group (65/124, 52.4%) was enrolled at the South African site. Pathogens were identified in similar proportions of the blood cultures of admitted infants (10.8%, 71/658) and those not admitted (9.7%, 12/124).

The median age of infants in the 0–6 day group was 2 days while the median age for those aged 7–59 days was 24 days (Table 1). There were more male than female infants in both groups, and more than half of infants (55%) aged 0–6 days and 75% of the older infants were born in health facilities. Twelve percent of neonates in the 0–6 day group were premature (estimated gestation age <37 weeks), whereas only 5.3% of the older infants were premature. Major reasons for referral for hospital management of infants varied by age group, although sepsis, hyperbilirubinemia and birth asphyxia were in the top 5 for both groups (Table 1).

Clinical Diagnoses of Infants with Positive Blood Cultures

Twenty-eight of 305 (9.2%) infants referred for hospital management who had blood cultures performed in the absence of

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│         8,889 infants enrolled           │
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│  1,437 infants classified as requiring   │
│       further hospital management         │
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│   782 infants had blood cultures done    │
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                    │───────────▶│  Contaminants    │
                    ▼            │    (n = 48)      │
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│  83 infants with probable   │
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│ 44 pathogens │  │  39 pathogens in │
│ in 0-6 day   │  │  7-59 day infants│
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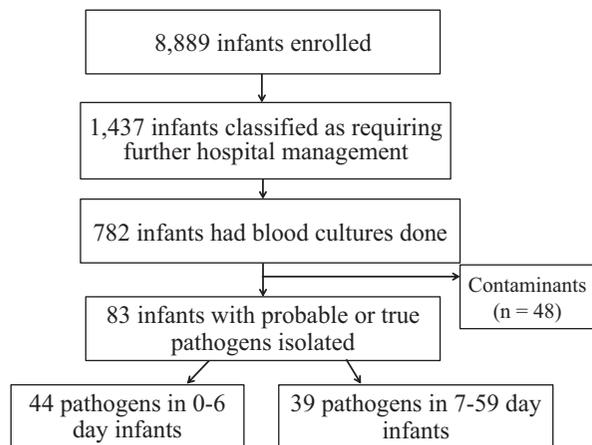
FIGURE 1. Study profile demonstrating the total number of infants enrolled, proportion referred for hospital management and proportions by age group with positive blood culture results.

any signs of infection had a positive culture; most of these were neonates in the first week of life (10.5%, 23/220). Focal infections (alone) were present in 33 infants of whom 4 had positive cultures (12.1%).

Systemic infection alone (sepsis, meningitis, pneumonia) in the absence of focal signs of infection was diagnosed in 381 infants, of whom 37 had positive cultures (9.7%). There were 63 infants with systemic illness associated with a focal finding, including 14 (22.2%) who had positive blood cultures.

Blood Culture Results

Blood culture results for all patients stratified by age group are presented in Table 2. *S. aureus* was the most commonly isolated organism. There was 1 group A streptococcus and no GBS isolated. Gram-negative bacilli were also commonly isolated. One infant in Pakistan had polymicrobial bacteremia due to *E. coli*, *Aeromonas hydrophila*, *Proteus mirabilis* and *Pseudomonas aeruginosa*; this infant died from overwhelming sepsis associated with an acute abdomen. There were no major differences in the rates of isolation of *S. aureus* or Gram-negative rods between the 2 age groups. *S. aureus* was predominantly isolated from infants in Ghana (15/36, 42%) (Table 3). In contrast, the distribution of other organisms was relatively, evenly spread among the sites.

The rates of positive cultures and distribution of pathogens were similar in infants who were premature (gestational age <37

weeks) versus term or who were delivered at home versus in the hospital (with the possible exception of more *Pseudomonas* spp. isolated from infants with home vs. facility-based deliveries; 4/33 vs. 0/50, $P = 0.01$).

Notably, the proportions of *S. aureus* positive cultures stratified by initial clinical diagnostic category were the following: 13/305 (4.3%) (no diagnosis of infection), 13/381 (3.4%) (sepsis diagnosis), 2/33 (6.1%) (focal infection) and 8/63 (12.7%) (focal plus systemic) ($P = 0.012$). These results point to a higher proportion of *S. aureus* isolates in the focal plus systemic infection group.

Antimicrobial Susceptibility Results

Since the study sites used different panels of antibiotics for susceptibility testing, the number of isolates tested for different antimicrobial agents varied. Among the Gram-positive isolates, *S. aureus* was most commonly tested (n = 45). Most *S. aureus* isolates were resistant to penicillin (88%, 22/25) and cotrimoxazole (66%, 21/32). Lower levels of resistance among the *S. aureus* isolates were found for erythromycin (19%, 8/43) and ciprofloxacin (14%, 3/22). Methicillin/oxacillin resistance was identified in 11% of the *S. aureus* isolates (4/37).

The susceptibility patterns of Gram-negative bacteria for which there were 2 or more isolates are presented in Table 4. Nearly all isolates were resistant to ampicillin or amoxicillin, first generation cephalosporins, chloramphenicol and cotrimoxazole. Resistance to second and third generation cephalosporins occurred in more than half of isolates. Similarly, a moderate proportion of isolates were resistant to gentamicin (17/39, 43%) and ciprofloxacin (11/31, 35%).

Clinical Outcomes of Infants With Bacteremia

The choice of empirical antimicrobial therapy varied from site to site. For those sites that systematically documented which antibiotics were used, the clinicians involved generally modified their choice of treatment after receiving the blood culture results. Broad-spectrum second and third generation cephalosporins with or without an aminoglycoside were used for Gram-negative infections while third generation cephalosporins or antistaphylococcal penicillins were used for treatment of *S. aureus*. Mortality rates were similar in admitted infants with positive (5/71, 7.0%) and negative blood cultures (42/557, 7.5%) (Table 2). Mortality rates were higher in the 0–6-day-old than the 7–59-day-old infants (38/383, 9.9% and 12/272, 4.4%, respectively). Excluding all infants whose blood culture isolate was *S. aureus*, in whom mortality was 2.8% (1/36), there still was no apparent association between bacteremia and mortality in either 0–6-day-old infants [3/20 (15%) vs. 34/343 (9.9%), $P = 0.46$] or in 7–59-day-old infants [1/19 (5.3%) vs. 11/241 (4.6%), $P = 0.89$].

Of the 12 bacteremic infants who were not admitted, there were 9 for whom day 3 outcome data were available. Six had

TABLE 1. Demographic and Birth Characteristics

Characteristic	Neonates Aged 0–6 d (n = 424)	Infants Aged 7–59 d (n = 358)
Age, median (d) (IQR)	2 (1–4)	24 (12–41)
Female	37%	41%
Delivery location: Home	45%	25%
Health facility	55%	75%
Prematurity*	12%	5.3%
Five main reasons for referral for hospital management	Hyperbilirubinemia (27%)	Sepsis (26%)
	Sepsis (24%)	Pneumonia/ALRI (16%)
	Birth asphyxia (23%)	Birth asphyxia (12.5%)
	Prematurity (10%)	Hyperbilirubinemia (12%)
	Low birth weight (2.8%)	Meningitis (3.6%)

IQR, interquartile range.
*Defined as estimated gestational age <37 weeks.

TABLE 2. Blood Culture Results Stratified by Age Group for All Infants

Isolate	0–6 d N = 44	Case Fatality Rate N (%)	7–59 d N = 39	Case Fatality Rate N (%)	Total N = 83	Case Fatality Rate N (%)
Gram-positive organisms						
Staphylococcus aureus	21	1/21 (4.8)	15	0/15 (0)	36	1/36 (2.8)
Group A streptococcus	1	0/1 (0)	0	NA	1	0/1 (0)
S. pneumoniae	1	0/1 (0)	1	0/1 (0)	2	0/2 (0)
Enterococcus faecalis	1	0/1 (0)	4	0/4 (0)	5	0/5 (0)
Gram-negative organisms						
Escherichia coli	4	1/3 (33)	3	0/3 (0)	7	1/6 (16.7)
Klebsiella spp.*	4	0/4 (0)	4	0/4 (0)	8	0/8 (0)
Acinetobacter spp.	4	0/4 (0)	5	0/5 (0)	9	0/9 (0)
Pseudomonas spp.	4	1/3 (33)	0	NA	4	1/3 (33)
Enterobacter spp.	1	0/1 (0)	5	0/5 (0)	6	0/6 (0)
Other Gram-negative bacilli†	3	1/3 (33)	2	1/2 (50)	5	2/5 (40)

*includes 4 isolates of *K. pneumoniae*, the other 3 were not identified to the full species level.
†includes 2 isolates of *Salmonella* spp. and 1 of each of the following: *Vibrio cholerae*, *Serratia marcescens* and *Alcaligenes faecalis*.
NA, not applicable.

TABLE 3. Blood Culture Results by Site (n = 83)

Isolate	Bangladesh N = 10	Bolivia N = 1	Chandigarh N = 20	Delhi N = 14	Ghana N = 21	Durban N = 6	Pakistan N = 11
Staphylococcus aureus	3	0	9	3	15	4	2
Group A streptococcus	0	0	0	0	0	0	1
S. pneumoniae	1	0	0	0	0	0	1
E. faecalis	0	0	2	1	0	1	1
E. coli	0	0	1	2	1	0	3
Klebsiella spp.	1	0	1	1	3	1	1
Acinetobacter spp.	2	0	4	3	0	0	0
Pseudomonas spp.	2	0	1	0	0	0	1
Enterobacter spp.	0	0	1	4	1	0	0
Salmonella spp.	1	0	0	0	1	0	0
Other Gram-negative bacilli*	0	1	1	0	0	0	1

*These included 1 isolate of each of the following organisms: *Alcaligenes faecalis*, *Vibrio cholerae* and *Serratia marcescens*.

TABLE 4. Antimicrobial Resistance Patterns of 40 Gram-negative Blood Culture Isolates, Displayed as Proportion Resistant*

Name	*E. coli* N = 7	*Klebsiella* spp. N = 8†	*Acinetobacter* spp. N = 10‡	*Pseudomonas aeruginosa* N = 3	*Pseudomonas* spp. N = 2§	*Enterobacter* spp. N = 8¶	*Salmonella* spp. N = 2	Total N = 40 (%)
Ampicillin/amoxicillin	5/6	7/7	5/5	NT	2/2	7/7	2/2	28/29 (96.6%)
1st generation cephalosporin	1/2	1/2	5/5	NT	2/2	4/5	1/1	14/17 (82.4%)
2nd generation cephalosporin	3/6	2/7	4/5	0/1	2/2	3/6	1/2	15/29 (51.7%)
3rd generation cephalosporin	4/6	3/4	6/9	0/2	2/2	3/7	1/1	19/31 (61.3%)
Chloramphenicol	3/3	3/5	1/2	0/1	2/2	2/2	2/2	13/17 (76.5%)
Ciprofloxacin	1/6	2/4	4/10	1/3	1/2	2/5	0/1	11/31 (35.5%)
Cotrimoxazole	4/4	4/6	2/2	NT	0/2	3/3	1/2	14/19 (73.7%)
Gentamicin	2/7	3/8	6/10	0/3	2/2	3/7	1/2	17/39 (43.9%)
Amikacin	1/6	2/3	4/8	0/2	NT	2/5	NT	9/24 (37.5%)
Meropenem/imipenem	0/4	0/3	2/8	NT	1/2	0/9	0/1	3/27 (11.1%)

*(number resistant/number tested); NT, not tested.
†Three specimens were fully identified as *K. pneumonia*.
‡One specimen was identified as *A. baumanni*, 3 as *A. anitratus* and 2 as *A. lwoffi*.
§One specimen was identified as *P. cepacia*.
¶One specimen was identified as *E. cloacae*.

improved, 2 had not improved and 1 was classified as sick and requiring urgent attention.

DISCUSSION

This multi-site study, which included 7 sites in 6 countries, enrolled a large number of young infants brought to health facilities for evaluation of a broad range of illnesses. Among infants requiring urgent hospital management, the main organisms isolated were *S. aureus* and several different species of Gram-negative bacilli. Notably the distribution of Gram-negative bacilli and Gram-positive organisms was similar between the 2 age groups: newborns 0–6 days and infants aged 7–59 days. This study was designed to emulate community sites with a wide spectrum of milder illnesses,

not only infections, but also illnesses such as jaundice as an isolated clinical sign and birth asphyxia, which have a low risk of bacterial infection. Even in children with a high likelihood of bacterial infection, the isolation rate was relatively low (~11%). Notably, a large summary of blood culture data from a tertiary care center in London found that 12% of 8904 cultures taken from neonates with suspected sepsis were positive[14] and a summary of bacteremia data from hospitalized children in Kilifi, Kenya, found that 12.8% of infants <60 days old that had blood cultures yielded pathogens.[15] Similar results were found at the same site in a study of community-acquired bacteremia in infants <2 months old with possible serious bacterial infection which found a prevalence of 9%.[16] These are remarkably similar to our isolation rate. However, it is possible that pre-treatment with antibiotics occurred in some of our patients before presentation to the study center and this might have reduced the rate of blood culture positivity.

Only slightly more than half of the infants who were admitted had blood cultures done even though this was supposed to have been done as part of the study protocol. In many cases, this was due to a decision by the study pediatrician that doing a blood culture was not necessary based on their admitting clinical diagnosis, which included conditions that were not due to infection such as severe jaundice in the absence of other clinical signs. Although these technically represented protocol violations, it is unlikely that these infants would have had positive cultures since many were admitted for management of diseases unlikely to be complicated by serious bacterial infections.

There are relatively few other studies from developing countries that have studied large numbers of neonates and infants with community-acquired bacteremia. A review of 63 studies, including 13 that focused on community-acquired infections, found that *Klebsiella* spp., *E. coli* and *S. aureus* were the most common isolates in the first week of life while *S. aureus*, GBS, *Streptococcus pneumoniae* and nontyphoidal *Salmonella* spp. were the most frequent isolates in infants ranging from week 2 of life to 90 days.[17] A previous WHO-supported study found that Gram-positive organisms, especially *S. aureus*, *S. pneumoniae* and *S. pyogenes* were present in 61% of blood culture isolates (n = 102).[18] This study found Gram-negative bacilli in 39% of young infants that cultures performed, with *E. coli* and *Salmonella* spp., the most commonly encountered Gram-negative isolates. A community-based study in Mirzapur, Bangladesh found a mix of Gram-positive isolates with *S. aureus* predominating and Gram-negative isolates in neonates.[11] Thus, all of these studies, many of which are included in the aforementioned review, found a similar range of pathogens to what we encountered in our study. The consistent identification of *S. aureus* as a possible pathogen in young infants might be secondary to horizontal transmission in facility-based deliveries and person-to-person transmission in home deliveries resulting in colonization and infection. Alternatively, *S. aureus* skin carriage coupled with inadequate site sterilization might have resulted in the inoculation of bacteria into the blood culture bottles or some of the positive cultures resulted from transient *S. aureus* bacteremia, which was not clinically significant. Consequently, the role of *S. aureus* as a true pathogen remains unclear.

One of the striking findings of our study was the paucity of Gram-positive organisms other than *S. aureus*, especially GBS. The latter is the most common Gram-positive pathogen reported for this age group in developed countries.[19] In contrast, it was rarely found in the previous Young Infant Study or the Mirzapur community-based study.[11,18] Our findings are thus consistent with several other studies that have shown a low prevalence of GBS[20]; this pathogen may be less commonly encountered in developing relative to resource-rich countries due to a lower prevalence of

maternal and neonatal colonization or colonization with less virulent strains which are less likely to cause invasive disease.[17,21] In developed countries, GBS became a dominant pathogen only in the 1960s.[22] Traditionally, the main species of streptococcus was group A, both as a pathogen for puerperal sepsis and for neonatal infections.[23] Improvements in hygiene may have contributed to this shift. However, it is also possible that newborns with sepsis due to GBS present as critically ill, especially in early onset neonatal sepsis, and they are at substantial risk of early mortality.[24] Since we excluded newborns requiring immediate cardiopulmonary resuscitation, some more critically ill newborns might have had early onset GBS sepsis. Furthermore, some neonates with GBS sepsis might have died before reaching the health facility, thus potentially explaining the absence of early and late onset GBS bacteremia in our study.

Resistance to inexpensive, widely used antibiotics (eg, cotrimoxazole, penicillin and ampicillin/amoxicillin) was common. These findings are consistent with a recent review which found that nearly half of *S. aureus* isolates were resistant to cotrimoxazole and a substantial proportion of *E. coli* isolates were resistant to cotrimoxazole and ampicillin.[25] A worrisome finding was the presence of methicillin resistance in 11% of *S. aureus* isolates. In the community-based Bangladesh study, 10% (1/10) of *S. aureus* isolates were oxacillin resistant.[11] Limited data from other studies of community-acquired bacteremia suggest that methicillin-resistant *S. aureus* is uncommon.[25] However, susceptibility results were only available for 5 isolates in that review in contrast to our study, which included methicillin resistance testing for 37 isolates. In contrast, a recent review, which highlighted the paucity of data on antimicrobial resistance patterns among neonatal sepsis pathogens, suggests that some locations in sub-Saharan Africa may be encountering increasing problems with methicillin-resistant *S. aureus*, based on evidence of ceftriaxone resistance.[26]

An additional finding of concern in our study was the presence of moderate to high levels of resistance of Gram-negative isolates to a number of different antimicrobial agents, including later generation cephalosporins, gentamicin and ciprofloxacin. Since the study eligibility criteria were designed to avoid inclusion of recently hospitalized newborns and thus exposure to nosocomial pathogens, the presence of multi-drug resistance in community isolates is of great concern. In view of these resistance patterns, less intensive community-based treatment regimens for newborn sepsis, which rely on fewer injections with gentamicin and oral amoxicillin, may not provide adequate antimicrobial treatment coverage.[27]

There are a number of limitations that merit comment. There were many infants referred for hospitalization who did not have blood cultures performed and thus we may have missed some episodes of bacteremia. The protocol excluded critically ill infants in need of immediate resuscitation, some of whom may have had infections that we missed. We do not have long-term outcome data for the infants who participated in the study. Since susceptibility testing was not done routinely at all sites and there is likely to be regional variation in resistance patterns, the value of the study to guide selection of antibiotics for treatment of sepsis is limited.

Given the broad array of different pathogens identified in newborns and young infants with community-acquired bacteremia, the high prevalence of resistance to commonly used front-line antibiotics and a suggestion of moderate levels of resistance to newer, more expensive antimicrobial agents, there is a need for tertiary care health facilities in resource-poor countries to have the capacity to perform blood cultures, identify pathogens and their susceptibility profiles. Improved microbiological capacity and regional surveillance of resistance patterns will help improve the management of community-acquired and hospital-acquired neonatal sepsis. At the primary health care level, the choice of antibiotic regimens for

neonatal sepsis needs to be guided by local resistance data, when available. In addition, therapy should consider the addition of antistaphylococcal coverage, especially when signs of skin or soft tissue infection such as omphalitis are present.[28]

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APPENDIX 1. Laboratory Methods and Quality Control Systems

Study Site	Microbiology (Culture) Method	Susceptibility Testing Method	Quality Control Measures	Comments
Dhaka, Bangladesh	Conventional culture in TSB (Oxoid) for blood cultures; sheep blood and MacConkey agar for plating; Mueller Hinton agar for antimicrobial susceptibility testing	Disc diffusion MIC (E-test) for specific antibiotics to which the organism(s) were resistant	Testing of media (internal QC) for: 1. Sterility 2. Ability to support growth with typical colony characteristics and biochemical reactions ATCC strains for antimicrobial susceptibility testing	Formal EQAS was not available
La Paz, Bolivia	Traditional blood culture system with brain heart infusion broth (BBL); subculture on sheep blood agar, MacConkey agar, for plating; identification of all Gram-negative bacilli with the API System (Biomérieux)	Disk diffusion (Kirby–Bauer) following the CLSI method using standard antibiotic discs (Mueller Hinton media)	Internal QC for: 1. Sterility 2. Ability to support growth with typical colony characteristics and biochemical reactions 3. Temperature recorded and maintained daily in the morning for all refrigerators and incubators ATCC strains for antimicrobial susceptibility testing External QC: Enrolled with INNLSA of the Ministry of Health and Sport of Bolivia	There was no automated system during the study period
Kumasi, Ghana	Traditional blood culture system with brain heart infusion broth	Disk diffusion (Kirby–Bauer)	ATCC strains for antimicrobial susceptibility testing	No automated system during the time period of the study
Chandigarh, India	Traditional blood culture in TSB and bile broth (Hi-Media, India) for blood cultures; subculture on sheep blood agar and McConkey agar plates; Mueller Hinton agar (Hi-Media) for susceptibility test	Disc diffusion susceptibility tests following the CLSI method using standard antibiotic discs (Hi-Media)	Internal QC Participated in WHO external QC (Peter Vandamme, Belgium) till 1998 (stopped by WHO) with 98% score	Automated BACTEC system was not available before 1998
New Delhi, India	Conventional culture in TSB (Difco) for blood cultures; sheep blood and MacConkey agar for plating; Mueller Hinton agar for antimicrobial susceptibility test	Disc diffusion	Testing of media (internal QC) for: 1. Sterility 2. Ability to support growth with typical colony characteristic 3. Positive/negative controls for biochemical reactions ATCC strains for antimicrobial susceptibility testing	Formal EQAS not available Participated in a CDC/WHO EQAS in another project during the study period
Karachi, Pakistan	Bactec 9240 instrument using Peds Plus bottles followed by isolation Identification of all Gram-negative rods was done using the API System and for Gram-positives using conventional biochemical tests[2]	Kirby–Bauer method *S. pneumoniae* MIC for penicillin checked by agar dilution method or E-test strip *Enterococci* screened for VRE using 16 μg vancomycin plate High level gentamicin resistance checked using oxoid 120 μg disc *S. aureus* screened for methicillin resistance using 30 μg cefoxitin disc MIC for ciprofloxacin and ofloxacin was checked for all nalidixic acid resistant *Salmonella* spp. by agar dilution method	**External QC:** • Enrolled with College of American Pathologists (CAP) External Quality Assurance Program **Internal QC:** • All plate and tube media quality controlled using ATCC strains • Daily QC preformed on all biochemical reagents before use • All typing antisera QC against ATCC strains when the new vial is reconstituted or used • Weekly QC maintained for all antimicrobial discs in use against ATCC strains • Temperature recorded and maintained daily first thing in the morning for all refrigerators and incubators • Daily QC maintenance recoded for Bactec instrument	
Durban, South Africa	Bactec 9240 utilizing Paeds Plus bottles Identification of Gram-negative rods by API system and Gram-positives by conventional culture	Kirby–Bauer method *S. pneumoniae* MIC for penicillin checked by agar dilution method or E-test strip High level gentamicin resistance checked by oxoid disc *S. aureus* screened for methicillin resistance using 30 μg cefoxitin disc	**Internal QC:** • All plate and tube media QC by ATCC strains • Daily QC on all reagents prior to use • All typing antisera QC against ATCC strains when new vial reconstituted • Weekly QC for microbial discs in use against ATCC strains • Daily QC maintenance recorded for BACTEC instrument	

EQAS, external quality assurance; QC, quality control; ATCC, American Type Culture Collection; MIC, minimum inhibitory concentration; API, analytical profile index.

APPENDIX 2. Members of the YICSS Group

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