Etiology of pneumonia, sepsis and meningitis in infants younger than three months of age in Ethiopia

[Etiology And Clinical Signs Of Serious Infections In Young Infants In Developing Countries: A WHO Collaborative Study]

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Methods. Within a multicenter study coordinated by WHO, an investigation of the etiologic agents of pneumonia, sepsis and meningitis was performed among infants younger than 3 months of age seen at the Ethio-Swedish Children's Hospital in Addis Ababa for a period of 2 years. Of the 816 infants enrolled 405 had clinical indications for investigation.

Results. There were a total of 41 isolates from blood cultures from 40 infants. The study showed that the traditionally known acute respiratory infection pathogen Streptococcus pneumoniae was most common in this extended neonatal age group, found in 10 of 41 blood isolates. Streptococcus pyogenes was a common pathogen in this setting (9 of 41 blood isolates), whereas Salmonella group B was found in 5 of 41 isolates. Streptococcus agalactiae, which is a common pathogen in developed countries, was absent. A study of the susceptibility pattern of these organisms suggests that a combination of ampicillin with an aminoglycoside is adequate for initial treatment of these serious bacterial infections, but the combination is not optimal for the treatment of Salmonella infections. Among 202 infants on whom immunofluorescent antibody studies for viruses were performed based on nasopharyngeal aspirates, respiratory syncytial virus was found in 57 (28%) infants, and Chlamydia trachomatis was isolated in 32 (15.8%) of 203 infants.
Ethiopia is one of the least developed countries in the world with an infant mortality rate ranging from 66 in urban areas to 192 per 1000 in rural lowlands. More than 50% of the infant mortality rate in this population results from deaths occurring before 2 months of age. Acute respiratory infections (ARI) account for 20 to 35% of deaths in Ethiopian infants. The EthioSwedish Children's hospital in the Ethiopian capital, Addis Ababa, where the present study was performed, pneumonia accounts for 11% of all admissions and 7% of hospital deaths. The hospital is a tertiary center providing both primary and tertiary care, with most patients coming from the low income community of Addis Ababa. Addis Ababa is 2800 meters above sea level. In a small pilot study conducted in preparation for this project, it was shown that apparently healthy infants younger than 3 months of age living at this altitude had a lower arterial hemoglobin oxygen saturation than infants at sea level [92 ± 2% (mean ± 2SD)]. The low oxygen saturation may predispose children in Addis Ababa with pneumonia to more frequent hypoxemic episodes, which probably worsens both morbidity and mortality.

There is a need to determine the microbial etiology of pneumonia, sepsis and meningitis in young infants for individual case management, as well as to define appropriate preventive and treatment strategies at a national level. In the management of infants between 1 and 3 months of age, there is a dilemma with regard to the most appropriate first line antibiotic treatment for serious infections. The organisms considered as possible etiologic agents in this age group could be the known neonatal pathogens such as Gram-negative enteric bacilli, Streptococcus agalactiae (group B Streptococcus) and Listeria monocytogenes or those affecting older infants like Haemophilus influenzae and Streptococcus pneumoniae. Furthermore most published studies on this subject are from developed countries. There are contradictory reports regarding the prevalence of S. agalactiae among neonates who have pneumonia, sepsis or meningitis in developing countries.

The present WHO-coordinated multicenter study on pneumonia, sepsis and meningitis among infants younger than 3 months of age was designed to define the clinical presentations and their laboratory correlates using a carefully standardized clinical examination and chest radiography, blood and cerebrospinal fluid cultures. This paper assesses the prevalence of positive bacterial cultures, including antimicrobial susceptibility patterns, among infants younger than 3 months of age, using strict blood and cerebrospinal fluid (CSF) culture procedures. During a part of the study period we investigated the prevalence of viruses as well as Chlamydia trachomatis in nasopharyngeal aspirates from the same patients.

**PATIENTS AND METHODS**

The details of the methods and procedures involved in the study are described elsewhere in this supplement. A trained research nurse screened all infants younger than 3 months of age who presented at the Ethio-Swedish Children's Hospital from August, 1991, to the end of July, 1993, after obtaining consent from the mother or the guardian. The triage nurse used a predefined set of clinical symptoms and signs to decide to enroll patients as well as criteria to exclude them from the study. After a detailed clinical examination by one of the pediatricians (LM, MT, SL, SK), the infants were investigated for etiologic agents. The same investigations were performed on a group of control patients who did not fulfill the criteria and who were selected randomly, 1 for every 10 cases investigated on clinical grounds. All enrolled patients were followed while
hospitalized or as outpatients for 1 week. Many of the patients received high dose intravenous penicillin G and gentamicin initially, and some of them received ceftazidime and amikacin based on susceptibility patterns and drug availability during the study period. For all patients oxygen saturation was measured initially and subsequently. Oxygen was administered when the arterial hemoglobin oxygen saturation was <90% with the use of nasopharyngeal catheters or nasal cannulas.

Bacteriological methods were as described in this supplement. Anaerobic cultures were included using unvented commercial brain-heart infusion blood culture bottles and incubating a blood agar subculture under anaerobic conditions. Blood was drawn from the antecubital vein, and 1 ml each was placed in a prefixed tryptic soy broth and brain-heart infusion, 1 ml of blood was used to determine white blood cell count and differential and a thick blood film was prepared when malaria was suspected. Two milliliters of serum were stored below -20°C for C-reactive protein estimations and serologic studies. Cerebrospinal fluid was taken for culture and analysis when meningitis was suspected clinically. Chest radiography was performed within 24 h of presentation for all cases and controls. Urine was collected, in periene bags in most cases, for culture and to check for the presence of antimicrobial activity.

Bacteriologic procedures followed the BOSTID manual with some modifications as described previously. Cultures that showed signs of growth were Gram-stained and subcultured onto blood and chocolate agar plates. Enterobacteriaceae were differentiated with API20E. Staphylococci were differentiated by the coagulase test. All strains were stored at -70°C and S. pneumoniae, H. influenzae and strains of streptococci were confirmed in a reference laboratory. Bacteria classically known to be pathogenic and growing in blood or CSF at any stage after inoculation in either of tryptic soy broth or brain-heart infusion bottles (S. pneumoniae, H. influenzae, Streptococcus pyogenes, S. agalactiae or Salmonella spp.) were considered significant without additional criteria. However, bacteria that are known to be normal flora of the skin or intestine like Staphylococcus aureus, enterococci, Escherichia coli, Klebsiella spp., Serratia spp., Pseudomonas spp., Acinetobacter spp. and Enterobacter spp. were considered pathogenic only if they grew in both bottles or, if only one bottle was inoculated, the organism grew in <48 h, or if the organism was found in both blood and CSF. If the organisms grew in one of two bottles within <48 h, they were classified as a "Maybe" group but not included in the main analysis. All coagulasenegative staphylococci, Bacillus spp., Candida spp. and Streptococcus viridans were considered contaminants irrespective of their growth pattern. The antimicrobial susceptibility was tested by the Kirby-Bauer disk diffusion method.

During part of the study period it was possible to collect nasopharyngeal washings and perform immunofluorescent antibody testing for virologic and chlamydial studies as detailed in this supplement. Respiratory syncytial virus (RSV), parainfluenza virus, influenza A and influenza B virus and adenoviruses were sought by immunofluorescence only. Nasal swabs were cultured in charcoal media to identify Bordetella pertussis. Swabs from eyes, umbilicus and skin were taken whenever clinically indicated and plated onto blood agar, chocolate agar and MacConkey agar. Eye swabs were also plated onto GC agar. A Venereal Disease Research Laboratories test was performed whenever congenital syphilis was suspected by the physician.
Prevalence of pathogenic bacteria were computed for each age group and final diagnosis. The proportion of infants with and without bacteremia was compared for outcome variables by chi square values. Ethical clearance of the study was obtained from the research and publications committee of Addis Ababa University and the Secretariat Committee on Research Involving Human Subjects at the World Health Organization.

**RESULTS**

Of 2298 infants triaged 816 (35.5%) were enrolled into the study. The remaining babies, except for 6 refusals, were not enrolled because they had 1 or more of the exclusion criteria. Among those enrolled 405 infants (49.6%) had 1 or more of the clinical criteria for investigation and thus were investigated. 35 (4.3%) of the 411 infants without the clinical criteria were randomly sampled for investigation. Three hundred seven infants (38% of those enrolled) were hospitalized, and there were 88 deaths (case-fatality rate, 11% of enrolled infants).

There were 41 isolates from blood and 8 from CSF (Table 1). Among the CSF isolates all except one also grew in blood. S. pneumoniae (pneumococcus) was isolated in 5 of the meningitis cases. Two of the pneumococcal meningitis cases died within 24 h of admission. Both had inability to suck, feeble, weak cry, blank dull stare, reduced movement and hypotonia when they were brought to the hospital. The patient with S. pyogenes meningitis had signs of overwhelming septicemia with necrotizing enterocolitis and died 2 days after admission. One patient with H. influenzae was also critically ill but went home against medical advice and probably died. S. pneumoniae and S. pyogenes were common isolates from blood. There were no S. agalactiae isolates (Table 1). The 10 infants with S. pneumoniae were spread throughout all age groups including neonates and infants in the second and third month of life. All S. pyogenes isolates were from infants <1 month old. Distribution of these bacteria by final diagnosis is shown in Table 2. S. pneumoniae and H. influenzae were associated with pneumonia, sepsis and meningitis, whereas S. pyogenes and E. coli were associated with sepsis alone. Salmonella group B isolates were associated with both sepsis and pneumonia. Klebsiella pneumoniae grew in 8 patients but all except 1 were excluded by the set criteria because the isolates either grew in one of the bottles only or grew after 48 h. Most of these patients had urinary tract or wound infections. H. influenzae was found in 3 cases only, 2 in the second month of life. Among the Gram-negative organisms, Salmonella group B and E. coli were most frequent. There were 2 infants with Staphylococcus aureus septicemia and 4 with B. pertussis from nasal swabs. Eight infants had purulent eye discharge, 6 of whom were positive for Neisseria gonorrhoea. A Venereal Disease Research Laboratories test was performed on 159 suspected infants, and 20 (12.5%) were reactive and treated as congenital syphilis.

**TABLE 1. Frequency of isolates of bacterial organisms from blood and CSF by age**
TABLE 2. Final diagnoses by individual bacterial organisms in blood and/or CSF

The overall number of isolates was small. Antimicrobial susceptibility testing showed that S. pneumoniae, H. influenzae, E. coli and S. pyogenes isolates were susceptible to the combination of penicillin G and gentamicin, the usual treatment for serious bacterial infections in the neonatal age group (Table 3). All Salmonella and Klebsiella isolates were resistant to ampicillin, chloramphenicol and cotrimoxazole. H. influenzae isolates were susceptible to all the antibiotics except cotrimoxazole. The Staphylococcus aureus isolates were sensitive to all antibiotics except penicillin G.

TABLE 3. Number of bacterial isolates from blood and CSF susceptible to commonly used antibiotics

A comparison of the initial assessment of infants with and without bacteremia is shown in Table 4. A high association with both bacteremia and fatal outcome was seen for the overall clinical assessment of severe illness by the physician, the clinical diagnosis of pneumonia and the clinical diagnosis of sepsis, based on the judgment of the clinician. A low oxygen saturation level on initial examination did not correlate with bacteremia. The neonates younger than 8 days of age were no more likely to have bacteremia than older infants.

TABLE 4. Comparison of bacteremic with nonbacteremic infants

Nasopharyngeal aspirates were collected from 202 cases and immunofluorescent antibody testing was performed for respiratory syncytial virus, influenza virus type A and type B, parainfluenza virus and adenovirus. Seventy-four (36.6%) infants were found to be infected, 57 (28.2%) with RSV. Parainfluenza virus, influenza viruses and adenovirus were also found. Studies for C. trachomatis were performed on 203 nasopharyngeal specimens; 32 (15.8%) were positive. A breakdown by age for RSV and C. trachomatis is shown in Table 5. Thirty-four (60%) of the RSV-positive cases were diagnosed as upper respiratory tract infections, and 15 (26%) were diagnosed as pneumonia. RSV detection was common in the rainy months, i.e. June, July, September and October. Ninety-six percent of all detected cases occurred in those 4 months.


<table>
<thead>
<tr>
<th>Age Group</th>
<th>RSV-positive</th>
<th>Chlamydia-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7 days</td>
<td>24</td>
<td>7 (28%)</td>
</tr>
<tr>
<td>1-4 wk</td>
<td>103</td>
<td>31 (30%)</td>
</tr>
<tr>
<td>5-8 wk</td>
<td>58</td>
<td>10 (41%)</td>
</tr>
<tr>
<td>9-12 wk</td>
<td>47</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>57 (28%)</td>
</tr>
</tbody>
</table>

**TABLE 5.** RSV and Chlamydia trachomatis positivity by indirect fluorescent antibody by age group.

**DISCUSSION**

In studies conducted in developed countries, the pattern of bacterial agents of neonatal sepsis and meningitis has been changing over recent decades. In the early part of this century, the dominant organism was S. pyogenes. By the 1950s, it was replaced by Staphylococcus aureus and E. coli, and in recent decades, E. coli and S. agalactiae have accounted for ~70% of all infections. Studies of the etiologic agents of sepsis in young infants in developing countries in the 1980s showed that Klebsiella, Staphylococcus aureus and E. coli were the dominant organisms. In contrast, the present study has shown that S. pneumoniae is a very important etiologic agent even in neonates. Klebsiella spp. and Staphylococcus aureus were uncommon. S. agalactiae was absent, even in newborns enrolled during the first few hours of life, whereas S. pyogenes was a dominant organism during the first month of life. Although H. influenzae was seen in small numbers, the fact that it was a causative agent in this age group is an important finding.

In previous studies from Ethiopia, it was shown that among 344 isolates from blood or CSF, 11% were caused by Klebsiella spp., 19% were caused by E. coli and 6% were caused by S. aureus. In Addis Ababa, in a study of fatal neonatal sepsis associated with amniotic fluid infection, the rate of early neonatal sepsis was estimated at 21.8 of 1000 live births and the common bacteria identified postmortem in the lungs and placenta were Ureaplasma spp. (26 and 44%, respectively), E. coli (20 and 12%), Acinetobacter spp. (16 and 11%), S. epidermidis (15 and 13%) and unclassified streptococci (11 and 10%). There are few investigations from developing countries of the etiology of pneumonia in infants younger than 3 months of age. In this study, which included infants with pneumonia, and which used strict techniques to isolate pneumococci, we found that pneumococci represent an important pathogen in this age group, causing both pneumonia and meningitis. Although known pathogens like pneumococci and S. pyogenes represent important causes of pneumonia, sepsis and meningitis, the rate of isolates of commensal species, especially coagulase-negative staphylococci, was quite high in this study. According to specific criteria developed by the research group, the latter were considered to be contaminants.

While small numbers limit the generalizability of our data, this study offers the opportunity to examine the appropriateness of recommendations generally followed in Ethiopia. In the WHO guidelines for management of pneumonia, sepsis and meningitis, it is stated that an infant younger than 2 months of age should be managed with penicillin G and gentamicin when these infections are suspected. Our data suggest that ampicillin and gentamicin would be more appropriate as coverage of Salmonella spp. improves. Although this sort of combination is appropriate at the rural health facility level, there is a need to include a second line antibiotic combination to cover the few resistant isolates that may not respond to this regimen. For part of the study period, amikacin and ceftazidime were available and were used when isolates were shown to be resistant to the initial combinations. We recommend that such a second line...
antibiotic regimens including third generation cephalosporins be available at the referral level for patients who do not respond to the first 48 h of treatment with the initial antibiotic combination. Referring a critical infant after management for 2 days in a rural or regional hospital requires a functioning referral system which is unfortunately not available in many areas of developing countries at the moment.

Cotrimoxazole is one of the drugs recommended in the WHO guidelines 19 for first line treatment of pneumonia in children older than 2 months of age. In this study the small number of isolates of H. influenzae was resistant to cotrimoxazole. Because the implications of in vitro resistance on treatment efficacy are not clear, more work is required before definitive conclusions about these findings can be made. Chloramphenicol is another drug recommended as second line antibiotic for pneumonia cases that do not respond to cotrimoxazole, but our study showed that S. pneumoniae and H. influenzae isolates were sensitive to chloramphenicol, whereas Salmonella group B and Klebsiella spp. isolates were resistant. Again there is a need for further study to determine the role of chloramphenicol as a second line drug.

The high frequency of RSV identification among our infants is consistent with previous results.20-22 Among subjects in whom RSV was detected 60% had upper respiratory tract infections and 26% had pneumonia. The descending order of frequency of detection of RSV, parainfluenza and influenza viruses is consistent with the results from Kenya and Uganda.23, 24 Our results as well as other investigators 25, 26 demonstrate the etiologic importance of C. trachomatis in ARI among young infants.

Our study has shown that among infants younger than 3 months with pneumonia, sepsis and meningitis, S. pneumoniae, S. pyogenes, Salmonella group B and E. coli are common isolates from blood and/or CSF. S. agalactiae was not isolated. The combination of penicillin G and gentamicin when administered for serious bacterial infections in this age group in our hospital will cover most organisms except Salmonella group B, but third generation cephalosporins should be made available at referral centers for cases not responding to first line therapy. RSV and C. trachomatis were most commonly isolated from nasopharyngeal aspirates of these infants.

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Key words: Etiologic agents; pneumonia; sepsis; meningitis; young infants; developing countries