Etiology of serious infections in young Gambian infants

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Abstract
Background. Despite improvements in infant mortality rates in many developing countries including The Gambia, neonatal mortality remains high and many neonatal deaths are caused by infection. The study described in this paper was conducted to determine the bacterial and viral etiology of serious infections in Gambian infants younger than 91 days old.

Methods. At a first level health facility 497 infants with symptoms that could indicate serious infection were enrolled, of whom 239 with 1 or more signs of serious infection and 55 with no signs were investigated, yielding 17 cases with positive bacterial cultures of blood and/or cerebrospinal fluid. At a nearby pediatric referral hospital 198 infants were seen and 182 were investigated, yielding 35 positive bacterial cultures.

Results. There were 15 culture positive cases of meningitis caused by Streptococcus pneumoniae (7), Streptococcus pyogenes (2), Enterobacter cloacae (2), Escherichia coli (1), Haemophilus influenzae type b (1), Streptococcus agalactiae (1) and Salmonella spp. (1). Six of these children died. Thirty-three infants without meningitis had positive blood cultures for Staphylococcus aureus (17), S. pneumoniae (3), Salmonella spp. (5), E. coli (3), other enterobacteria (4) and S. agalactiae (1), of whom 14 died. Nasopharyngeal aspirates from 438 children were investigated for common respiratory viruses. Respiratory syncytial virus was found in 51, influenza A in 46, influenza B in 22, parainfluenza in 26 and adenovirus in 16. Respiratory syncytial virus and influenza A isolates were found most frequently toward the end of the wet season. Nasopharyngeal carriage of S. pneumoniae and H. influenzae was studied in 320 infants.
recruited during the first year. Of these 184 (58%) were positive for S. pneumoniae and 141 (44%) were positive for H. influenzae, 18 of which were type b. Infants with a bacterial isolate from blood or cerebrospinal fluid were more likely than the rest to die, whereas those with a viral isolate were less likely to die.

Conclusions. The most important causes of serious infections in young Gambian infants are Staphylococcus aureus, S. pneumoniae and Salmonella spp.

INTRODUCTION

During the past two decades there has been a dramatic reduction in infant and childhood mortality in The Gambia. Mortality rates in children younger than 5 years of age have fallen from ~50% in the period 1951 to 1975 to 17% in recent years.1, 2 Despite these marked improvements there has been less change in the neonatal mortality rate which was 50 to 85 per 1000 live births in the period 1951-1975 and presently is 41 per 1000 live births. If deaths in the first 3 months of life are considered, the mortality rate is 54 per 1000 live births. Thus in The Gambia, as in other developing countries, neonatal and young infant mortality is becoming the major contributor to childhood mortality. The causes of this mortality have not been well-studied. Investigations conducted in The Gambia during the past 10 years, using the technique of postmortem questionnaire, have suggested that about one-half of all neonatal deaths, and a higher proportion of deaths in the second and third months of life, are caused by infections.2, 3 Although published studies from developing countries indicated that Klebsiella spp. and Staphylococcus aureus are the most important causes of neonatal sepsis in developing countries, anecdotal evidence in The Gambia suggested that Streptococcus pneumoniae and perhaps Haemophilus influenzae are more important causes of community-acquired infections in very young infants.

The etiology of serious infections in young infants is likely to vary according to the level within the health service at which a study is conducted. The majority of published series relate to infections detected at a tertiary referral center. These are likely to comprise a mixture of infections in referred cases, who have passed through one or more stages of the health care system before reaching the tertiary hospital, and hospital acquired infections. Thus, the etiologic spectrum of cases detected at a tertiary referral center may be very different from that of cases detected at a first level health facility or in a study based in the community. In The Gambia, postmortem questionnaire studies have shown that 68% of deaths in infants younger than 3 months of age occurred in infants who had not reached any conventional health care provider during their final illness (A De Francisco, personal communication). This is likely to be the case in many other African countries. An effective public health strategy aimed at reducing deaths from infection in early infancy should include a combination of effective treatment at the tertiary level, effective treatment and appropriate referral procedures at the first level health facility, early treatment in the community and prevention. At present preventative measures focus on improved perinatal care, but in the future specific measures such as maternal immunization against specific organisms may become available.
The present study was designed to address the question of etiology at two levels, the tertiary
hospital and the first level health facility. A third component, the etiology of infection in the
community, was abandoned because of practical constraints. This study formed part of the
WHO-sponsored multicenter study of serious infections in very young infants.

PATIENTS AND METHODS
The study was divided into two parts that were conducted in two consecutive years. The first part
was conducted at the outpatient department of the Medical Research Council (MRC) Hospital in
Fajara, a periurban area ~15 km from Banjul, the capital of The Gambia, a small developing
country in West Africa. This facility provides first level medical care for the population in the
surrounding area and for a number of families from other areas who choose to use the facility.
During the period September, 1990, through September, 1991, all children younger than 91 days
of age who presented to this facility were triaged by a field worker following the guidelines
described in an accompanying paper in this supplement. These guidelines resulted in all infants
with any sign that could be the result of infection being eligible for enrollment in the study. All
eligible infants whose mothers or guardians gave verbal consent were enrolled. A standard
history and examination was performed by one of the study pediatricians (OOO, MJM, EKM). If
a child had one or more criteria for laboratory investigation, they were subjected to full
laboratory study. In addition every fifth child who did not fulfill these criteria was investigated.

A venous blood sample was drawn under aseptic conditions for culture, hematologic
examination and storage of serum. A chest radiograph was performed. Urine was collected,
whenever possible by the clean catch technique, while blood was being drawn. If this approach
was not successful, a urine bag (Portex Ltd., Kent, UK) was applied. Nasopharyngeal aspiration
for bacterial culture and virology was performed using a 10 French gauge suction catheter
attached to a mucus collection device (Sterilin Medical Products, Teddington, UK) which was
connected to a mechanical suction apparatus. The mucus obtained was rinsed out with 1 ml of
phosphate-buffered saline.

During the second year of the study (January to December, 1992), infants younger than 91 days
of age who presented to the only pediatric referral hospital in The Gambia, The Royal Victoria
Hospital (RVH) in Banjul, who fulfilled the triage criteria and for whom consent was given,
were enrolled in the study. History, examination and investigations were then performed as
described above by one of the study pediatricians (MW, OOO, AP). During the first year of the
study, a small number of infants were recruited at RVH while we were endeavoring to conduct
the two components of the study concurrently; this was later abandoned for practical reasons.

After evaluation infants were treated as inpatients or outpatients by the study physician and
followed up 2 to 4 weeks later. Children who failed to turn up at follow-up appointments were
traced wherever possible and encouraged to come to the clinic.

During the course of the study, a control group of infants presenting to the health centers for
vaccination was recruited for virologic studies. These children were matched by age and health
center of registration with infants investigated and were recruited within 1 month of enrollment of the infant investigated. A nasopharyngeal aspirate was performed on each of these children.

Data were entered with Dbase III and Epi-Info software; analysis was conducted with SAS statistical software.

**LABORATORY METHODS**

Bacteriologic methods were standardized between sites and are described in the accompanying paper in this supplement. H. influenzae and S. pneumoniae were serotyped by latex agglutination using type-specific antisera obtained from Murex Diagnostics (Dartford, UK) and Statens Seruminstitut (Copenhagen, Denmark) for H. influenzae and S. pneumoniae, respectively. During the first year of the study nasopharyngeal aspirates were cultured for H. influenzae and S. pneumoniae.

In addition to seeking Mycoplasma spp. and Ureaplasma spp. at the site, a random selection of 59 nasopharyngeal aspirates were sent to the reference laboratory (L Duffy, Department of Microbiology, The University of Alabama at Birmingham). These specimens were inoculated directly into a specially prepared transport medium and kept at -70°C until they were transported to the reference laboratory in dry ice, within 90 days. At the reference laboratory they were thawed and inoculated into specialized broths for the isolation of Ureaplasma and Mycoplasma (10B and SP4, respectively). Broth cultures showing evidence of growth were inoculated onto specialized solid media and incubated in 5% CO2 at 37°C for 2 weeks. Species were identified by colonial morphology.

In addition to the work done to isolate viruses, Mycoplasma spp. and Ureaplasma spp., nasopharyngeal aspirates were inoculated onto bacitracin chocolate agar and gentamicin blood agar plates, which were incubated at 35°C in 5 to 10% CO2 for 24 to 48 h for the selective isolation of H. influenzae and S. pneumoniae. Nasopharyngeal swabs were inoculated onto cephalexin transport medium at the bedside and further cultured on cephalexin charcoal agar incubated for 7 days at 35°C in high humidity for isolation of Bordetella pertussis.

Capsular polysaccharide antigens of H. influenzae type b and S. pneumoniae were sought from inoculated blood culture medium and urine by latex agglutination with the use of Slidex Meningite-kit 5 (BioMérieux, France). Urine was also tested by countercurrent immunoelectrophoresis with Hib antiserum and S. pneumoniae Omniserum (Statens Serum Institut). Urine samples were kept at -20°C and tested for bacterial antigens within 2 weeks after boiling, filtration (HAW P 2500; Millipore, Watford, UK) and concentration (25×) with a Minicon B-15 concentrator (Amicin Div., Beverly, MA).

Respiratory tract viruses were detected in nasopharyngeal aspirates by rapid indirect immunofluorescence (IIF) and by cell culture. On delivery to the virology laboratory, specimens were homogenized and centrifuged at 350 × g for 10 min. The supernatant fluid was removed for inoculation into cell culture plates and tubes. The pellet was then washed by centrifugation and spread onto microscope slides. The slides were fixed and stained with IIF reagents for respiratory syncytial virus (RSV), influenza A, influenza B, parainfluenza (types 1, 2 and 3 combined),
measles and adenovirus, with the use of specific antisera provided by WHO. Positive and negative controls for each virus were included with each batch. The supernatant fluids were inoculated into 24-well plates of Vero, NCI-H292, HEp-2 and MRC-5 cells (American Type Culture Collection, Manassas, VA) for virus isolation. Tubes of Vero cells were also inoculated. The cultures were incubated for 14 to 42 days and examined for cytopathic effects. Viruses isolated were identified by IIF with the use of the six specific antisera mentioned above. Enteroviruses and rhinoviruses were identified by their reactions in the supernatant fluids, chloroform sensitivity and acid stability tests, but were not typed.

RESULTS

During the first year of the study, which was conducted at the MRC Hospital, 497 infants were enrolled in the study (Table 1). Of these 239 had 1 or more signs of sepsis and were investigated. A further 55 who did not have signs of sepsis, but were randomly selected, were also investigated. A total of 174 infants were investigated as possible cases of sepsis at the Royal Victoria Hospital (RVH), Banjul, 155 of them during the second year of the study. Twenty-four infants were seen at RVH who did not fulfil the criteria for investigation, of whom 8 were investigated. Thus a total of 695 ill infants were studied, of whom 413 fulfilled the criteria for investigation and a further 63 were randomly selected to be investigated. Of the 697 children enrolled 64 died, all of whom had fulfilled the criteria for investigation. Virologic studies were performed on 428 of the 476 children investigated and on 333 controls from the community.

### TABLE 1. Features of those infants enrolled in the study who fulfilled the criteria for investigation (Group A), those who did not but were randomly investigated (Group B) and those who were not investigated (Group C)

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<thead>
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<th>Group</th>
<th>Features</th>
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<tr>
<td>A</td>
<td>413</td>
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<tr>
<td>B</td>
<td>63</td>
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<td>C</td>
<td>697</td>
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Bacteriology results. There were 15 cases of cerebrospinal fluid (CSF) culture-positive meningitis, 11 of whom were also blood culture-positive. The distribution of organisms found in these children is shown in Table 2. Of the 7 infants with S. pneumoniae meningitis 2, ages 45 and 47 days, died. A third child was discharged with neurologic sequelae and died 2 months later. One 50-day-old child from whose blood and CSF S. pneumoniae was isolated and who also had septic arthritis on admission had a normal CSF cell count. This child made a good recovery. The younger of the 2 children with S. pyogenes meningitis was admitted initially as a case of neonatal pneumonia and treated with penicillin and gentamicin. She deteriorated the following day, when the diagnosis of meningitis was made; she died soon after. Two very young infants had meningitis caused by Enterobacter cloacae: the 12-day-old infant died the day after admission, whereas the 5-day-old infant appeared to make a good response to treatment, but deteriorated and died 11 days after admission. The infant with S. agalactiae (group B Streptococcus) meningitis had marked respiratory distress on presentation because of associated pneumonia. His blood culture grew Streptococcus epidermidis only.
Thirty-eight infants had septicemia without meningitis (Table 3). Seven of the 17 infants (41%) with S. aureus septicemia had significant skin rashes on presentation, mainly infected scabies, a similar proportion to that seen in the remaining infants (43%). Only 2 of the 7 infants had temperatures \( \geq 38.0^\circ \text{C} \). Three of the four infants with S. aureus septicemia who were less than 2 weeks old died. Four infants with S. aureus septicemia were among the 55 who were randomly sampled. All 4 had significant skin rashes and none were febrile. All 3 infants with S. pneumoniae septicemia had evidence of pneumonia with grunting and lower chest wall indrawing. A 7-day-old infant with S. pneumoniae septicemia, who weighed 1.74 kg and was hypothermic and tachypneic on admission, had clinical evidence of meningitis, but the lumbar puncture was traumatic and sterile. He died on the day of admission. Five children had Salmonella infection, 2 with type B organisms. The only child with Salmonella septicemia to survive was an 80-day-old infant who had pneumonia. Of the 4 infants with other Enterobacteriaceae infections, the only one to survive was a child with Klebsiella pneumoniae who also had influenza A infection. The other 3 died within 1 day of admission. One had clinical evidence of meningitis but was too ill for lumbar puncture.

There were 15 cases in which S. epidermidis was isolated from both blood culture bottles within 48 h. Because the clinical features of those children were no different from those of the children who were blood culture-negative, and because all infections were community-acquired, these were regarded as probable contaminants and not included in the analysis.

Urine samples were collected from 305 infants by suprapubic aspiration (32) clean catch (32) or perineal bag (241). Twenty children had urinary tract infections, defined as 10 or more white blood cells per high power field with a pure growth of pathogenic organisms from bag (16), suprapubic (2) or clean catch (2) specimens. The organisms grown were E. coli (10), K. pneumoniae (4), Candida albicans (5) and Proteus mirabilis (1). The median age was 59 days (range, 8 to 81), and the median temperature was 37.6°C (range, 36.3 to 40.6). All the children recovered well except for one who was discharged against medical advice, still ill.

Ureaplasma spp. and Mycoplasma spp. were not isolated at the site. However, from the 59 nasopharyngeal specimens transported to the reference laboratory Ureaplasma urealyticum was
isolated from 6. The median age was 68 days (range, 23 to 84 days), and 5 of the 6 children presented with respiratory symptoms. In only 1 was another pathogen isolated (parainfluenza virus) and all 6 made uneventful recoveries, only 2 having required hospital admission.

During the first year nasopharyngeal aspirates from 320 infants were cultured for H. influenzae and S. pneumoniae. Of these 141 (44%) were positive for H. influenzae (18 for H. influenzae type b) and 184 (58%) were positive for S. pneumoniae. The rate of positive cultures by age is presented in Figure 1.

![Figure 1. Proportion of infants under 91 days of age carrying Streptococcus pneumoniae, all H. influenzae and Hib, by age. Numbers in parentheses, number of infants studied in each age group.](ovidweb.cgi?View+Image=00006454-199910001-00007|FF1&S=IDNJHDKKEGMO00D)

Virology results. Four hundred thirty-eight infants underwent nasopharyngeal aspiration for virology (Table 4). One hundred sixty-one viruses were identified in specimens from 128 (29%) infants. Thirty-four of the viruses were identified in specimens from 24 (39%) of the 61 children who did not have an indication for investigation but were randomly investigated. During the same period 91 viruses were identified among 333 matched control infants from the community (27%). RSV was identified in 2 clusters, each in the wet season. This is consistent with more recent data from The Gambia which shows RSV to occur in annual epidemics at the end of the wet season. Influenza A was identified most frequently during the same periods (Fig. 2). In 22 cases 2 viruses were identified. The most frequent combinations were RSV and influenza A (5 cases), RSV and adenovirus (4 cases) and influenza A and influenza B (4 cases). There were 4 cases in which 3 viruses were identified and 1 case in which 4 viruses were identified.

![Table 4. Viruses identified in nasopharyngeal aspirate specimens](ovidweb.cgi?View+Image=00006454-199910001-00007|TT4&S=IDNJHDKKEGMO00D)

![Fig. 2. Number of infants by month [abscissa, starting September (S) and ending December (D)] from whom respiratory syncytial virus and influenza A virus were identified during the study period. The number of children investigated by quarter is indicated above the middle month for each quarter. In September, 1990, 27 children were investigated.](ovidweb.cgi?View+Image=00006454-199910001-00007|FF2&S=IDNJHDKKEGMO00D)
There were 4 cases in which viruses were identified in children with proven bacterial infections. One 12-day-old child with blood and CSF positive S. pneumoniae infection also had RSV. A 41-day-old child with influenza A and RSV had Staphylococcus aureus isolated from blood culture, and a 64-day-old child with influenza A had K. pneumoniae isolated from blood culture. A 5-day-old infant with influenza A had Enterobacter spp. septicemia and died soon after admission.

**DISCUSSION**

The present study describes the bacterial and viral etiology of community acquired infections in young Gambian infants. S. agalactiae (group B Streptococcus) and E. coli, usually regarded as the 2 most important causes of neonatal meningitis, contributed only 2 of the 15 cases of meningitis, whereas S. pneumoniae emerged as the most important cause of meningitis in young Gambian infants, causing 7, possibly 8 cases. Three of the cases of invasive S. pneumoniae disease were in infants younger than 2 weeks of age, emphasizing the importance of this organism as a true neonatal pathogen. The organism most frequently isolated from blood culture was S. aureus which was isolated from the blood of 18 infants (1 with S. pyogenes meningitis). As only six of those infants had rectal temperatures of 37.5°C or more and 7 had significant skin rashes, it could be argued that these isolates were contaminants. However, 6 of the 18 infants died, suggesting that the blood culture isolates were indeed significant.

The finding of six serious infections with Salmonella spp. organisms (five fatal, four in infants younger than 1 month of age) is disturbing as the usual antibiotic regimen selected for the treatment of serious neonatal infections in developing countries (gentamicin plus penicillin) is not effective against Salmonella spp. This raises the question of whether ampicillin should be specifically recommended for first line treatment of neonatal sepsis, cefotaxime should be recommended, or whether chloramphenicol, in appropriate doses, still has a role in the management of serious infections in very young infants in developing countries. The importance of Salmonella spp. as a pediatric pathogen in The Gambia has been emphasized in a recent study of pneumonia and sepsis in older infants. Several studies of bacterial meningitis from West Africa have identified Salmonella spp., particularly Salmonella typhimurium, as a major cause of Gram-negative meningitis, particularly in young children. Although it has been reported in some series of neonatal sepsis, its importance as a neonatal pathogen and the therapeutic implications have not been highlighted before.

It is of interest that only 2 infections with S. agalactiae (group B Streptococcus) were identified in this study. The catchment area for the two hospitals used in the study covers ~12 000 births per year. If the study is assumed to cover both hospitals for a period of 1 year, and if only one-third of cases of neonatal sepsis present for medical care, this would still represent a low rate of S. agalactiae infection (0.5/1000 live births) compared with industrialized countries where rates of 1 to 4 per thousand live births are reported. Only 3 studies from developing countries have demonstrated S. agalactiae infections in young infants. In other studies this organism has been notably absent. We have previously shown that S. agalactiae carriage is as common in Gambian women as in women from developed countries. Twenty-two percent of women in labor were positive on rectal or vaginal swab. The proportion of infants colonized was similar. Apparently Gambian infants are protected from S. agalactiae disease, possibly by maternal antibody acquired transplacentally or with breast milk.
This study has demonstrated the frequency of infection with common respiratory viruses in young Gambian infants. Children presenting to the first level health facility (MRC Hospital) were more likely to have a virus and less likely to have a bacterial isolate from blood or CSF than those who presented to the referral facility (RVH). This is the reason for the larger number of viral isolates in the first year of the study when recruitment was from the MRC Hospital. This finding is consistent with the belief that serious illness in young infants is more likely to be of bacterial origin, whereas less serious illness is more likely to be of viral origin. Indeed in our study infants from whom a virus was identified were significantly less likely to die than those from whom no virus was identified (5 of 128 vs. 41 of 310, P < 0.05, Fisher's exact test). Despite the smaller number of viral isolates in the second year, it is still evident that in both years of the study RSV was found most frequently during the wet season (July to October). Influenza was found throughout the year, but more frequently during the wet season. There were only 4 cases of mixed bacterial and viral infections, 3 of them involving influenza A.

The rapid acquisition of carriage of H. influenzae and S. pneumoniae during the first 3 months of life is consistent with the findings of Gratten et al. in Papua New Guinea.15 Given this high carriage rate it is not surprising to see S. pneumoniae emerge as an important pathogen in this age group. Thirteen percent of the H. influenzae isolates from the nasopharynx were type b, and the only case of invasive H. influenzae disease was caused by type b. Carriage rates for H. influenzae type b (Hib) are high in The Gambia16, 17 and the peak age incidence for invasive Hib disease is ~6 months,18 so it is not surprising to find Hib in nasopharyngeal specimens from infants younger than 3 months of age.

This study has demonstrated that, in very young Gambian infants with mild illness, common respiratory viruses such as RSV, influenza A and B, parainfluenza and adenovirus were frequently encountered and usually associated with a good outcome. In those with severe illness, the most important bacterial pathogens found were S. pneumoniae, Staphylococcus aureus and Salmonella spp. S. pyogenes and E. coli were found infrequently.

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REFERENCES


Key words: Neonatal sepsis; meningitis; pneumonia; Streptococcus pneumoniae; group B Streptococcus; Salmonella spp.; respiratory syncytial virus; influenza virus; developing countries; Africa

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