The Aetiology of Purulent Meningitis in Highland Children: A Bacteriological Study

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SUMMARY

Of 155 highlands children with purulent culture-positive meningitis studied from March 1980 to September 1984, 84% were aged twelve months or less and 92% were infected with either Haemophillus influenzae, Streptococcus pneumoniae or both organisms. Other pathogens were Neisseria meningitidis (8 isolations), Streptococcus pyogenes (2 isolations) and Klebsiella species (1 of each). Among H. influenzae isolates, serotype b strains predominated (83%) and most (96%) belonged to biotype I or II. Infections due to non-b haemophili included serotype a (9 strains), serotype f (1 strain) and non-serotypable variants (3 strains). Of 67 S. pneumoniae strains 22% were resistant to benzylpenicillin, with minimal inhibitory concentrations of 0.1 - 1.0 µg/ml. The commonest serotypes were types 5 (11 isolates), type 7 (9 isolates) and types 2, 6 and 46 (6 of each). No resistance to chloramphenicol was detected in either H. influenzae or S. pneumoniae and only one of 56 strains of H. influenzae was insensitive to betalactam antibiotics. The known case fatality rate in this study was 37%. More children with pneumococcal infection died (46%) than those with haemophilus infection (30%), though the difference was not statistically significant; 79% of all deaths occurred in children aged less than twelve months. There is an urgent need for H. influenzae and S. pneumoniae vaccines that are effective in young children.

INTRODUCTION

Meningitis is responsible for less than 1% of all hospital admissions in Papua New Guinea (PNG) (1). However, from 1977 to 1981 this infection caused 5.0 to 6.5% of all hospital deaths, two-thirds of which occurred in children four years old or less. In the 13-year period from 1970 to 1982, 192 of 840 children admitted to Goroka Base Hospital with acute meningitis died, a case fatality rate of 23%. In the same period meningitis accounted for 15% of child deaths at Goroka Hospital; it was second only to pneumonia as a cause of death.

The only published study of purulent meningitis in Papua New Guinean children is that of Biddulph et al. (2) in 1968, which dealt principally with the clinical features of the disease. Although some laboratory data were presented, cerebrospinal fluid (CSF) isolates were not differentiated serologically and sensitivity to antibiotics was not reported. The present investigation was undertaken to determine the agents responsible for acute bacterial meningitis in children in the highlands, to define the epidemiological types within the predominant groups and their quantitative in vitro response to commonly used antibiotics, and to monitor changes in aetiology and type distribution. The study period extended from March 1980 to September 1984.
METHODS AND MATERIALS

CSF from 155 children admitted to Goroka Hospital with culture-positive meningitis was studied. These patients represented 41% of all cases diagnosed during the study period. The ages of patients at presentation and, where possible, the outcome of the infection were recorded. No attempt was made to define the duration of illness before admission, the length of stay in hospital, nutritional status or long-term sequelae.

Most CSF specimens were cultured on the date of collection but on occasion they were stored at 4-6°C before culture. Both *H. influenzae* and *S. pneumaticae* survive well in CSF at refrigeration temperature (3). The samples were Gram-stained and primary cultures were made on both chocolate and horse blood agar and incubated for up to 72 hours at 35°C in an atmosphere enriched with 5-10% carbon dioxide.

*H. influenzae* strains were identified by Gram stain, colony morphology on chocolate agar, and dependence for growth on haemin and nicotinamide adenine dinucleotide. From January 1983, isolates were tested for their inability to excrete porphyrins (4). An attempt was made to serotype all strains by slide agglutination using *H. influenzae* antisera a t(Wellcome Reagents Limited, Beckenham, England). The substrates ornithine, urea and tryptophane, in conjunction with the Mitis system (BBL Microbiology Systems, Cockeysville, Maryland), were used to type all strains biochemically by the method of Kilian (5) as modified by Back and Oberhofer (6). Alpha haemolytic colonies resembling *S. pneumaticae* were tested for optochin sensitivity and serotyped by the quelling reaction with antisera from the Statens Serum Institut, Copenhagen. Beta haemolytic streptococci were identified by bacitracin sensitivity and coagglutination (“Phadebact”, Pharmacia Diagnostics AB, Uppsala, Sweden) and *Neisseria* and *Klebsiella* isolates by standard methods (7).

Quantitative agar dilution susceptibility testing to several antibiotics was performed by the method of Amend et al. (8). Minimal inhibitory concentrations (MIC) of benzylpenicillin or ampicillin to all strains of pneumococci and most strains of *H. influenzae* were determined. Leterly, the MIC of chloramphenicol for both *S. pneumaticae* and *H. influenzae* was assayed using the “Adastin” breakpoint system (Mast Laboratories Limited) with antibiotic concentrations of 0.2, 2.0 and

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<tbody>
<tr>
<td><em>H. influenzae</em></td>
<td>17</td>
<td>16</td>
<td>15</td>
<td>16</td>
<td>13</td>
<td>77</td>
<td>49.4</td>
</tr>
<tr>
<td><em>S. pneumaticae</em></td>
<td>15</td>
<td>15</td>
<td>12</td>
<td>11</td>
<td>14</td>
<td>67</td>
<td>42.9</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>5.2</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
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<tr>
<td><em>S. agalactiae</em></td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td><em>Klebsiella</em> spp</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.6</td>
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<td>Total</td>
<td>38</td>
<td>34</td>
<td>27</td>
<td>30</td>
<td>27</td>
<td>156</td>
<td>100.0</td>
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234
4.0 µg/ml. All H. influenzae isolates were tested for beta-lactamase activity using chromogenic cephalosporin (Glaxo Research Limited, Greenford, Middlesex, England).

RESULTS

Bacteriology

The CSF from 155 children yielded 156 significant isolations (Table 1). The major causative agents were H. influenzae (49%) and S. pneumoniae (43%). Other pathogens included N. meningitidis (8 isolations), S. pyogenes (2 isolations) and S. agalactiae and Klebsiella species (1 of each). A dual infection occurred in one infant with both H. influenzae serotype b, biotype II, and S. pneumoniae type 6. The incidence of meningococcal meningitis seen during the first year of the study was significantly higher than in the subsequent four years ($X^2$ with Yates correction = 4.58, 0.02 < p < 0.05). In 1980, 5 (13.5%) of 38 children yielded N. meningitidis while latterly only 3 isolations were made from 117 patients.

Of 77 H. influenzae strains 64 (83%) were serotype b. The remainder comprised type a (9 strains), type f (1 strain) and non-serotypable (NST) variants (3 strains). Table 2 lists the serotype and biotype of 73 isolates. Most (96%) belonged to biotype I or II, the latter accounting for 70% of serotype b organisms.

The serotype distribution and benzylpenicillin sensitivity of 67 S. pneumoniae strains are shown in Table 3; 19 serotypes were detected, the commonest being type 5 (11 isolations), type 7 (9 isolations), and types 2, 6 and 46 (6 isolations of each), together comprising 57% of all strains. An isolate from a two-month-old infant could not be serotyped although an unequivocal reaction was gained with Pool E antiserum (types 10, 12, 33, 39). In all, 22% of the pneumococcal isolates were partially resistant to penicillin, with MICs in the range 0.1 - 1.0 µg/ml. Of 77 H. influenzae isolates 56 were tested quantitatively against either benzylpenicillin or ampicillin. Only one isolate, an NST biotype III betalactamase-producing strain, was penicillin-resistant with an MIC of > 16.0 µg/ml. None of 22 strains of H. influenzae or 22 strains of S. pneumoniae showed reduced sensitivity to chloramphenicol.

<p>| TABLE 2 |
|-----------------|--------|--------|--------|--------|--------|
| <strong>SEROTYPE AND RELATIONSHIP TO BIOTYPE OF 73 STRAINS OF H. INFLUENZAE ISOLATED FROM CSF OF CHILDREN WITH ACUTE MENINGITIS</strong> |</p>
<table>
<thead>
<tr>
<th>Serotype</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>b*</td>
<td>17</td>
<td>42</td>
<td>0</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>f</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>NST**</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>49</td>
<td>1</td>
<td>2</td>
<td>73</td>
</tr>
</tbody>
</table>

* four strains were not biotyped
**NST: nonserotypable
TABLE 3

SEROTYPE DISTRIBUTION AND BENZYLPIenicillin SENSITIVITY
OF 67 STRAINS OF S. PNEUMONIAE ISOLATED FROM CSF OF
CHILDREN WITH ACUTE MENINGITIS

<table>
<thead>
<tr>
<th>a Benzylpenicillin MIC (µg/ml)</th>
<th>Serotype distribution b</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.05</td>
<td>1, 2(6), 5(11), 6(2), 7(9), 8(3)</td>
<td></td>
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<td></td>
<td>12(4), 18, 23(2), 27(2), 45(4)</td>
<td></td>
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<tr>
<td></td>
<td>46(6), E</td>
<td></td>
</tr>
<tr>
<td>0.</td>
<td>10, 24, 33, 35</td>
<td>4</td>
</tr>
<tr>
<td>0.2</td>
<td>6(4), 24, 35</td>
<td>6</td>
</tr>
<tr>
<td>0.5</td>
<td>14, 22, 23</td>
<td>3</td>
</tr>
<tr>
<td>&gt;0.5</td>
<td>14, 23</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>67</td>
</tr>
</tbody>
</table>

* aMIC: Minimal inhibitory concentration
* b( ): Number of strains per serotype

Age at presentation

The age of the children ranged from two days to ten years: 64% were six months old or less, 20% were aged six to twelve months and 16% were older than one year. The ages of two children were not recorded. Figure 1 relates the ages at infection to the causative agents. Infants were more susceptible to pneumococcal meningitis in the first three months of life (62% of all cases occurred in this age group), while haemophilus meningitis predominated (72%) in children aged four to eight months. Five neonates aged from two to thirteen days were seen. Included in this group were 2 of the 3 cases of nonserotypable *H. influenzae* infection, 2 due to *S. pneumoniae* type 1 and 12, and the only *S. agalactiae* infection.

Mortality

Of the 155 children studied 58 are known to have died, a case fatality rate of 37%; 79% of deaths occurred in children aged less than twelve months. However, 34 (22%) patients absconded and their outcome is unknown. Although more children with pneumococcal meningitis died (46%) than those with haemophilus disease (30%), the difference is not statistically significant (*X*² with Yates correction = 3.49, 0.05 < *p* < 0.10). Four cases of pneumococcal infection due to type 45 strains were seen; all were aged two years or older and all died. The mean age of these children was significantly higher (48 months) than that of other children (8 months) who died of pneumococcal infection. All cases caused by *S. pyogenes* (2 patients) and *Klebsiella* (1 patient) died. Only 1 of the 8 children with meningococcal infection died, but 4 absconded. Case fatality rates of patients with haemophilus disease due to type b (28%), type a (33%) and NST strains (33%) did not differ significantly.

DISCUSSION

The results of this study show that *H. influenzae* and *S. pneumoniae* cause most cases of purulent meningitis in children in the highlands. The proportion of cases caused by *N. meningitidis* decreased from 13.5% of all isolations identified in 1980 to 2.5% in 1981-84. An earlier study in 1964-67 reported *N. meningitidis* in 53% of 68 cases of culture-positive...
The predominance of type b H. influenzae was not surprising. This serotype has genuine invasive properties and, in healthy children in industrialised societies, is responsible for up to 95% of acute systemic disease caused by H. influenzae. In the current study, non-type b strains comprised 17% of the haemophilus isolates, of which nine were serotype a. A literature review conducted by Sutton et al. concluded that, after type b, type a haemophili are the commonest cause of invasive infections, particularly in infants (13). A more recent report has implicated type a haemophili in 2% of 544 cases of purulent meningitis in Africa (14). Type a strains were also recovered from 3 of 18 Apache Indian infants with invasive disease: 2 had meningitis and 1 bacteraemic pneumonia (15). Other reports of sporadic cases of type a disease have appeared from time to time (16-18). The greater invasiveness of type a haemophili may be related to their immunochemical structure, which is very similar to type b strains. These serotypes differ only in the neutral sugar (ribose or glucose) component (13). Sutton et al. (13) recently studied the comparative virulence of the six capsular types of H. influenzae by exposing each to the bactericidal effect of antibody-free complement. Type a organisms were the most resistant of the non-type b strains and types d and e were killed more rapidly than other serotypes.

Type f and NST H. influenzae have occasionally been implicated in meningitis (19-25). The upper respiratory tract is densely and persistently colonised with H. influenzae in over 95% of young highlands children: over 70% harbour NST variants and, among encapsulated carriage strains, type f is common (26). Both type f and NST haemophili have been isolated from lung tissue and blood of PNG children with acute pneumonia (27). H. influenzae meningitis is commonly associated with pneumonia in PNG (2). The invasion of blood from colonised lung tissue in highlands children with acute pneumonia has been
reported (27) and development of meningitis by this route has been shown experimentally (28). In the current study, 1 of the 3 infants with NST haemophilus meningitis had bacteremic pneumonia due to an NST variant with the same biotype (biotype II). Pneumonia was also present in the child with type f meningitis.

Less common pathogens included S. pyogenes (2 cases), and S. agalactiae and Klebsiella spp (1 case of each). Those with S. pyogenes meningitis were infants aged one and eight months and both died. Systemic infection with S. pyogenes following virus infections is well documented. An epidemic due to respiratory syncytial virus was peaking in the Asaro Valley at the time both children were hospitalised (P. Phillips, personal communication). Although neither had nasopharyngeal secretions examined for respiratory viruses one infant had moderate pneumonia. The case of S. agalactiae meningitis occurred in a neonate aged two days who survived. This organism typically causes neonatal sepsis and is a commensal of the adult female genital tract (29). Meningitis due to Klebsiella species is uncommon. Studies elsewhere report a high mortality (11, 30). The only case seen in the current investigation was an infant aged three months who died.

Of the 67 pneumococcal isolates in this study, 22% showed reduced sensitivity to benzylpenicillin. This is significantly lower than in pneumococci cultured from PNG children with acute pneumonia (27) (x^2 with Yates correction = 14.53, p < 0.001). It has been shown that highly invasive serotypes such as types 2, 5, 45 and 46 are rarely carried by PNG children (26). Their exposure to sublethal concentrations of penicillin, which encourages the emergence of resistant mutants, is therefore minimal (26, 31). In the current study, such serotypes contributed more than one half of all pneumococcal isolates. A further finding of interest was the isolation of type 45 pneumococci from four older children, all of whom died. The recovery of an encapsulated nonserotypable pneumococcus with one or more antigens common to group 10 organisms has been confirmed by Professor R. Austrian, Department of Research Medicine, University of Pennsylvania. This isolate represents a hitherto unrecognised S. pneumoniae serotype and was isolated from the CSF and blood of an infant with meningitis and bacteremic pneumonia. Antigens from 78% of the S. pneumoniae strains isolated are constituents of the 23-valent pneumococcal vaccine currently under trial in the Southern and Eastern Highlands Provinces.

Only one penicillin-resistant strain of H. influenzae was detected. The low prevalence of resistant haemophilus in PNG has been reported (32). The apparent difference in the response of S. pneumoniae and H. influenzae to penicillin is unexplained. However, the MICs of penicillin of both penicillin-resistant pneumococci and penicillin-sensitive H. influenzae fall within the same range, 0.1 - 1.0 μg/ml.

Of 73 haemophili that were biotyped 70 (96%) belonged to types I or II. A further virulence factor of H. influenzae, associated with biotypes I and II irrespective of serotype, has been suggested (33). Because of the preponderance of type b strains in this study, and the near exclusive association of both carriage (34) and invasive serotype b haemophilus with biotypes I and II in PNG, little can be contributed to this hypothesis. No significant difference in mortality caused by biotype I or II strains of type b H. influenzae occurred. However, 5 of 9 cases of serotype a meningitis were due to biotype II variants and 3 of these children died. Of the 2 infants with biotype II NST infections 1 died.

The high case fatality rate of 37% in this study was probably due to the fact that most children presented for treatment very late in their illness. Furthermore, only children with culture-positive meningitis were included: patients with partially treated culture-negative meningitis have a lower mortality. Our 155 patients represented 41% of all cases of meningitis admitted to Goroka Hospital Children’s Ward during the study period, when there was an overall fatality rate of 28% in children with meningitis. Reports from other developing countries have recorded high case fatality rates in children with meningitis: 30% of 130 children died in a prospective study in Ibadan,
Nigeria (11) and retrospective analysis of 402 cases of bacteriologically confirmed meningitis in African children showed a fatality rate of 58% (35). As in the present study, workers in Africa (10, 11), England (36) and Australia (37) have reported a higher fatality rate in children from pneumococcal meningitis than from meningitis caused by H. influenzae or N. meningitidis.

Our finding that a high proportion of isolates of S. pneumoniae are relatively insensitive to penicillin in PNG supports the use of chloramphenicol for the treatment of meningitis in children, and suggests the need for strenuous efforts to be made to reduce the widespread overuse of procaine penicillin in PNG. Because of the very high mortality in PNG children from meningitis and pneumonia caused by H. influenzae and S. pneumoniae, there is an urgent need for the development of H. influenzae and S. pneumoniae vaccines that are effective in children less than 12 months of age.

ACKNOWLEDGEMENTS

We are grateful to Helen Gratten for typing the manuscript and to the Ward 4 staff of the Goroka Base Hospital for assistance in specimen collection.

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