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The etiology of lobar pneumonia in the Gambia*

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Sixty-four patients who had been admitted to hospital in the Gambia with acute lobar pneumonia were investigated. Lung aspiration proved to be the most effective method of establishing a bacterial etiology, and Streptococcus pneumoniae *was the pathogen isolated most frequently from patients irrespective of age. Among children,* Haemophilus influenzae, *either singly or in combination with another bacterial agent, was an important cause of pneumonia. Of 13 isolates of* H. influenzae *two were of serotype a, while four others were non-capsulated. All isolates of* S. pneumoniae *and* H. influenzae *were sensitive to penicillin.*

Acute gastrointestinal and acute respiratory infections are two of the most important causes of mortality and morbidity in most developing countries, including the Gambia. While considerable progress has been made in determining the etiology of acute diarrhoeal diseases and in defining a strategy for their treatment and prevention, less attention has been paid to acute respiratory infections. Indeed, in many developing countries the etiology of acute respiratory infections in children is unknown, and the relative importance of bacterial and viral causative agents is undetermined (*1*).

The difficulties of establishing a firm bacterial etiology for patients with pneumonia are well known. Culture of sputum is of uncertain value, while blood culture provides a definitive diagnosis but is positive in only 20–30% of patients. Although analysis of sputum for capsular polysaccharide antigens may be of diagnostic value in adults (*2–4*), samples of sputum are difficult to obtain from children. Alternative approaches to the diagnosis of acute respiratory infections in children include the use of invasive diagnostic techniques such as lung aspiration (*5–9*) and sensitive serological tests for the detection of bacterial antigens in biological fluids (*10–12*). As a preliminary survey, prior to a more comprehensive study of respiratory infections among children in the Gambia, we investigated the etiology of pneumonia in a group of hospitalized patients using the following techniques: lung aspiration, blood culture, and detection of antigen in serum.

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MATERIALS AND METHODS

Patients

During the period December 1982 to January 1984 a total of 207 patients with pneumonia were admitted to the Medical Research Council Hospital, Fajara, Gambia. Of these, 64 who had had symptoms for less than 7 days' duration, for whom clinical and/or radiological evidence of pulmonary consolidation was available, and who agreed to be investigated by lung aspiration, formed the basis of the study. Patients suspected to have post-measles pneumonia and those with severe malnutrition complicated by pneumonia were excluded. None of the study patients was known to have had prior treatment with antibiotics.

Lung aspiration

Lung aspiration was carried out in the region of maximum pulmonary consolidation using a 21-gauge needle attached to a 10-ml syringe containing 1–2 ml Mueller-Hinton broth.[a] Patients' skin was first cleansed with 70% (v/v) isopropyl alcohol. As soon as the skin had been penetrated, the plunger of the syringe was withdrawn slightly, the needle inserted quickly into the lung, and removed without a pause. The aspirate in the syringe was then transferred to a sterile tube, which was centrifuged for 10 minutes in a bench-top centrifuge. The centrifugate was used to prepare smears for Gram-staining, to inoculate chocolate agar (5% defibrinated horse blood in blood agar base No. 2[a]), blood agar (5% sheep blood in Columbia base[a]), and Robertson's cooked meat culture medium. There were no complications arising from the lung aspiration.

[a] From Oxoid, Basingstoke, Hampshire, England.

Bacteriology

Primary culture plates obtained from lung aspirates were incubated for 48 hours in a candle jar until negative. Subsequently, Robertson's cooked meat culture medium was subcultured for 48 hours onto blood agar incubated in a mixture of air and carbon dioxide, anaerobically, and onto chocolate agar. Venous blood from patients was inoculated into nutrient broth with liquoid (sodium polyanethyl sulfonate) and into tryptone soya broth with liquoid. Subcultures were performed at intervals of 24 and 48 hours, and 7 days. Subsequent growths of colonies were identified by standard techniques. *Haemophilus influenzae* was biotyped using the method described by Kilian (*13*) involving indole, urease (EC 3.5.1.5), and ornithine decarboxylase (EC 4.1.1.17) activity; *H. influenzae* and *Streptococcus pneumoniae* were serotyped by coagglutination or countercurrent immunoelectrophoresis (CIE) using type-specific serum.[b, c]

Antibiotic sensitivity testing

The sensitivity of the method was determined by comparative disc diffusion (Stokes' method) using *Staphylococcus aureus* NCTC 6571 as a control and the following antibiotics in the disc: penicillin 0.25 IU, chloramphenicol 10 μg, tetracycline 10 μg, sulfamethoxazole 25 μg, and trimethoprim 1.25 μg. Isolates showing reduced sensitivity were further investigated by the antibiotic-incorporated, agar-plate dilution methods.

Detection of bacterial antigens

Sera were analysed for capsular polysaccharide antigens by CIE using pneumococcal omniserum[d] and *H. influenzae* type b antiserum[e] (*14*). The sensitivity of this technique was determined for 14 purified polysaccharides of *S. pneumoniae* (types 1, 2, 3, 4, 6A, 8, 9N, 12F, 14, 19F, 23F, 25, 7F, and 18C, Danish classification).[f] The sensitivity of the assay ranged from 5 μg/ml for type 14 polysaccharide to 100 ng/ml for types 3, 6, 7, and 9 polysaccharide.

Selected sera were examined for pneumococcal antigens by a micro-ELISA technique. Briefly, a γ-globulin precipitate of pneumococcal omniserum diluted to a concentration of 10 μg/ml in carbonate buffer (pH 9.6) was adsorbed by incubation for 16 hours at 4 °C onto the cavities of a 96-well microtitration plate.[g] Unfilled binding sites were blocked by incubation for 1 hour at 37 °C with PBS-Tween containing 5% newborn calf serum. After washing in PBS-Tween, 200 μl of test serum was added to duplicate wells and the plate incubated for 2 hours at 37 °C. After further washing in PBS-Tween, captured antigen was identified by omniserum conjugated to alkaline phosphatase using the method described by Avreamas (*15*). After a final washing with PBS-Tween, captured conjugate was detected by incubation with the substrate tris(*p*-nitrophenyl) phosphate for 1 hour at 37 °C. The optimum conditions, sensitivity, and experimental variation of the test methods were established using purified pneumococcal polysaccharides as controls. The sensitivity of the assay ranged from 50 ng/ml (type 4, 8, 18, and 23 polysaccharides) to 2.5 ng/ml (type 3 polysaccharide).

[b] Type-specific serum for *H. influenzae* was obtained from Difco, Detroit, MI, USA.

[c] Type-specific serum for *S. pneumoniae* was obtained from Statens Seruminstitut, Copenhagen, Denmark.

[d] Staten Seruminstitut, Copenhagen, Denmark.

[e] Difco, Detroit, MI, USA.

[f] Samples of polysaccharides were kindly supplied by Merck, Sharp & Dohme, Hoddesdon, Hertfordshire, England.

RESULTS

Of the 64 patients with pneumonia in the study, 51 were less than 10 years of age. A bacterial etiology was established for a high proportion of both adults and children (Table 1). Lung aspiration was the most useful diagnostic procedure, and the lung aspirates from 29 of the 51 children (57%) were positive, as were those from 7 of the 13 older patients (54%) (Table 2). Blood cultures were positive for 14 children (27%) and for 2 adults (15%). The sera from only 6 of the 64 patients (9%) were positive by CIE; however,

[g] Nunc, Kamstrup, Roskilde, Denmark.

Table 1. Relationship between age and the established bacterial etiology of pneumonia among patients in the study

Age group (years)	No. of patients	
	Total	With positive bacteriological diagnosis
<1	15	11 (73)[a]
1-1.9	16	9 (56)
2-2.9	9	6 (67)
3-3.9	4	3 (75)
4-4.9	5	5 (100)
5-9	2	1 (50)
10-14	0	0
15-19	5	2 (40)
⩾20	8	6 (75)
Total	64	43 (67)

[a] Figures in parentheses represent percentages.

Table 2. Efficacy of diagnostic procedures in establishing a bacterial cause of pneumonia among patients, by age group, in the study

Procedure	No. of patients aged		Total
	<10 years	≥10 years	
Culture of lung aspirate only	19	6	25
Culture of blood only	4	1	5
Culture of lung aspirate and blood	10	1	11
CIE of serum alone[a]	2	0	2
Total diagnosed	35	8	43
Total studied	51	13	64

[a] Countercurrent immunoelectrophoresis.

for 2 patients this was the only way of diagnosing pneumococcal disease, and antigen was detected by this method in the sera of 2 of the 6 culture-positive cases of *H. influenzae* type b infection.

Eighteen sera, 10 from patients with culture-proven pneumococcal pneumonia and 8 from undiagnosed patients, were tested for *S. pneumoniae* antigen by enzyme-linked immunosorbent assay (ELISA); 8 of the culture-proven and 3 of the undiagnosed cases were positive. Patients who were positive only by ELISA were not included with the proven cases. Only 2 of the 8 sera from patients with culture-proven pneumococcal pneumonia that were positive by ELISA were also positive by CIE. Ninety-seven of 100 control sera obtained from patients with no clinical evidence of pneumococcal pneumonia were negative for *S. pneumoniae* antigen by ELISA, and the 3 positive sera came from patients with pronounced splenomegaly.

Table 3. Distribution of bacterial etiology in 43 of 64 patients, by age group, in the study

Bacterial etiology	No. of patients aged		Total
	<10 years	≥10 years	
Streptococcus pneumoniae alone	22	7	29
Haemophilus influenzae alone	8	0	8
S. pneumoniae and *H. influenzae*	2	1	3
S. pneumoniae, *H. influenzae*, and *Branhamella catarrhalis*	2	0	2
Staphylococcus aureus	1	0	1
No diagnosis	16	5	21
Total	51	13	64

Table 4. Distribution of serotypes of *Streptococcus pneumoniae* isolated from patients in the study

Serotype	No. of isolates
5	8
1	5
6	4
14, 19, 46	2 each
2, 8, 18, 22, 23, 32	1 each

Diagnoses

For 43 of the 64 patients in the study the results obtained by at least one diagnostic procedure are shown in Table 3. *S. pneumoniae* and *H. influenzae* were the pathogens identified most frequently.

S. pneumoniae was identified as an etiological agent in 34 of the 64 patients studied (53%) and in 34 of the 43 patients (79%) for whom a bacterial etiology was made; for 29 of the latter patients it was the sole pathogen identified. For 21 patients the diagnosis was established only by lung aspiration. *S. pneumoniae* was the pathogen identified most frequently in patients of all age groups; however, there was no correlation between age and the likelihood of a positive blood culture.

Twenty-eight pneumococcal isolates were serotyped and a further infection was serotyped by direct CIE of serum (Table 4). Type-5 *S. pneumoniae* was identified most frequently. All patients with type-5 pneumococcal pneumonia presented from January to May 1983, but there was no geographical clustering of cases. Because of the small number of isolates studied, no statistical correlation between serotype and age can be made, but types 6, 19, and 46 were isolated only from children under 2 years of age. No serotype was overrepresented in isolates obtained from blood cultures.

Antibiotic sensitivity patterns were determined for 28 pneumococcal isolates. Two type-5 isolates were resistant to both chloramphenicol (minimal inhibi-

tory concentration (MIC), 16 mg/l) and tetracycline (MIC, 16 mg/l). These isolates were obtained from 2 patients admitted only 4 days apart. A further three isolates (type 6, 14, 23) were resistant to tetracycline (MIC, 8–16 mg/l) but not to chloramphenicol. All isolates were sensitive to penicillin (MIC, <0.12 mg/l), erythromycin, and co-trimoxazole.

H. influenzae was isolated from 13 of the 64 patients (20%) and from 13 of 43 patients (30%) for whom a bacterial etiology was made. Isolation of *H. influenzae* was age-related, being isolated from 11 of 49 children under 5 years of age but from only 2 of 15 older patients. This difference was, however, not statistically significant. For eight patients *H. influenzae* was isolated alone, for a further three in association with *S. pneumoniae*, and for two others in association with *S. pneumoniae* and *Branhamella catarrhalis*. One of the last-mentioned patients, an infant of 4 months, died.

Seven of the 13 isolates of *H. influenzae* were of serotype b, two were of serotype a, while four were non-capsulated. All four non-capsulated isolates were obtained by lung puncture alone, while four of the seven serotype-b isolates were obtained from blood. Eight of the nine capsulated isolates were of biotype 1 and one was of biotype 4. Two of the four non-capsulated isolates were of biotype 2, while one each was of biotype 3 and biotype 5, respectively. All *H. influenzae* isolates were β-lactamase negative and sensitive to penicillin (MIC, 0.25–1 mg/l), ampicillin, chloramphenicol, and co-trimoxazole.

DISCUSSION

The study confirmed that lung aspiration is the most sensitive technique for establishing a cultural diagnosis of bacterial pneumonia in patients with consolidation, although a combination of lung aspiration, blood culture, and determination of serum antigen was required to achieve maximum diagnostic yield. Although lung aspiration was not associated with any morbidity in the study, it can cause haemoptysis and pneumothorax (*6*, *9*) and it should, therefore, only be used with patients who can be kept under close observation. A simpler and safer method is required if pneumonia is to be diagnosed among patients who are to be managed in the community. Detection of capsular polysaccharide antigens in sputum by simple immunological techniques, such as CIE or coagglutination, can be of diagnostic value for adults (*2*, *4*) but is not applicable for children; furthermore, detection of antigen in serum using these simple immunological methods is too insensitive to be of diagnostic value (*6*). We have, therefore, explored the use of a more sensitive ELISA technique for detecting pneumococcal capsular polysaccharide antigens in the serum of patients with pneumonia, and preliminary results are encouraging. A high proportion of positive reactions was obtained for patients with culture-positive pneumococcal pneumonia; however, positive reactions, which were probably false positives, were obtained from three patients with splenomegaly and may have been caused by a rheumatoid factor binding to rabbit γ-globulin. An ELISA assay may prove to be of value in diagnosing pneumococcal pneumonia in situations where lung aspiration is impracticable.

S. pneumoniae and *H. influenzae* were isolated either alone or together from 34 of the 64 patients (53%) studied, confirming the importance of these bacteria as the predominant etiological agents of pneumonia in hospitalized patients in developing countries. As expected, *H. influenzae* was isolated more frequently from children than from adults. In the case of children our results are very similar to those obtained, also by lung aspiration, in a recent study of the etiology of pneumonia among children admitted to hospital in Papua New Guinea (*9*). *B. catarrhalis*, an organism of uncertain pathogenicity, was isolated by lung aspiration in conjunction with *H. influenzae* from children in both studies.

S. pneumoniae was the most frequent cause of pneumonia associated with pulmonary consolidation in Gambian adults and children admitted to hospital, as reported previously in Nigeria (*6*, *14*, *16*). Type 5 was the predominant serotype, and pneumococci of this serotype have been recorded previously as important etiological agents of pneumococcal meningitis and pneumonia in Nigeria, Senegal, and the Gambia (*17*, *18*) but are rare elsewhere, although the reason for this is not clear. Type 5 pneumococcal polysaccharide was not included in the original 14-valent pneumococcal polysaccharide vaccine but it is included in the new 23-valent replacement.[h]

H. influenzae, either alone or in combination with *S. pneumoniae* was the second most important cause of pneumonia in the study sample. When *H. influenzae* was isolated alone, all but one of the isolates were capsulated, and 75% of them were of serotype b. As has been reported in other studies, the majority of isolates of this serotype were of biotype 1 (*19*). Two isolates of *H. influenzae* serotype a were obtained by lung aspiration and, in a subsequent study of invasive *H. influenzae* infection in Gambia, a further three isolates of type-a *H. influenzae* were obtained from patients with pneumonia and two from patients with meningitis (R. A. Wall, unpublished data). Type-a *H. influenzae* has been associated with paranasal sinusitis (*20*), but only occasionally with invasive disease in industrialized countries (*21*). In Papua New Guinea, only 19% of the isolates

[h] Merck, Sharp & Dohme, Hoddesdon, Hertfordshire, England.

of *H. influenzae* obtained from children with pneumonia were of serotype b, the remainder being either other serotypes or non-typable. Thus, in developing countries non-type-b *H. influenzae* may be an important cause of pneumonia and other invasive diseases.

The overall mortality of patients in our study was only 3%, but children with post-measles pneumonia and severe malnutrition were excluded, because of the higher morbidity from lung aspiration among them. Nevertheless, the overall mortality among the 207 patients with pneumonia admitted to the hospital during the study period was only 5%. This figure probably reflects the ease of access of patients in Banjul and its environs to medical care, and is probably not representative of the situation in the country as a whole. All isolates of *S. pneumoniae* and *H. influenzae* were sensitive to penicillin, which can thus be used safely to treat pneumonia in Gambian patients of all ages.

The patients included in our study were highly selected in that they were sufficiently ill to warrant admission to a busy hospital and because they had clinical or radiological signs of pulmonary consolidation. Analysis of outpatient diagnoses over a 1-year period in the hospital revealed that 214 adults and 648 children were treated for pneumonia: there were clinical signs of pulmonary consolidation among 73% of the adults and 44% of the children. The etiology of pneumonia in those patients who exhibited no evidence of consolidation has not yet been established.

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RÉSUMÉ

ÉTIOLOGIE DE LA PNEUMONIE LOBAIRE EN GAMBIE

Soixante-quatre cas de pneumonie lobaire aiguë hospitalisés en Gambie (Afrique de l'Ouest) ont fait l'objet d'une étude. L'aspiration s'est révélée la technique la plus efficace pour la détermination d'une étiologie bactérienne et le micro-organisme le plus souvent isolé, quel que soit l'âge, a été *Streptococcus pneumoniae*. Chez l'enfant, *Haemophilus influenzae*, soit seul, soit associé à une autre bactérie, s'est avéré une cause importante de pneumonie. Sur 13 isolements de *H. influenzae*, deux étaient du sérotype a tandis que quatre autres étaient non encapsulés. Tous les isolements de *S. pneumoniae* et de *H. influenzae* se sont montrés sensibles à la pénicilline.

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