Acta Paediatrica Hungarica, 27 (2), pp. 97—105 (1986)

Pneumococcal Infections During Childhood: Serotyping of Pneumococcal Strains from Forty-six Childern

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The results of serotyping of forty-six strains of pneumococci isolated from children aged 3/12 to 14 9/12 years and diagnosed as having pneumonia, meningitis, primary peritonitis, otitis media, lymphadenitis, osteomyelitis, bacteraemia and conjunctivitis are presented.

The results of serotyping and the frequency distribution of the detected serotypes were compared to the particular diagnoses established and the age at which the subjects involved had become ill. Questions of epidemiology and possibilities of immunoprophylaxis are discussed. Finally, the occurrence of pneumococci that are resistant to antibiotic agents are discussed since an isolated strain was found to show reduced susceptibility to penicillin G.

In spite of present day possibilities of therapy with antibiotics, infections with pneumococci (Streptococcus pneumoniae) still present major medical problems [27]. This particular fact and reports describing the occurrence of strains of pneumococci that were observed to be resistant to penicillin and other antibiotic agents [2, 13, 14] resulted in problems studied intensively in the last years.

There are few recent studies of results of typing of pneumococci isolated from diseased children [22]. This paper presents initial results of our studies of the subject.

Patients and Methods

The studies conducted by us included strains of pneumococci that were isolated between April, 1980, and January, 1984, from patients at the Children's Hospital and Clinic of Paediatric Surgery, Karl Marx University at Leipzig. The strains were obtained from blood cultures, cerebrospinal fluids (CSF), pleural and peritoneal fluids, ear and eye swabs, as well as abscesses and bone aspirates. Preliminary identification was carried out by bile solubility testing. Resistance determinations were made using the standard agar diffusion test [37].

The strains were freeze-dried and transferred for typing to the Laboratoire de Bactériologie-Virologie de l'Université Lyon I, France.

The capsular swelling reaction was used for serological determination with the aid of antipneumococcic sera (Statens Serum Institute, Copenhagen, Denmark; 9 pool sera, A through I, 46 type or group sera). The Danish nomenclature was used for the designation of types and groups. The clinical and laboratory data were considered for the arrangement or classification of diseased children in diagnostic groups. In one child where pneumococci were detected in the blood culture it was not possible to obtain significant evidence of local mani-

festation of the infection; that is why it was diagnosed as bacteraemia.

For statistical considerations, use was made of the U-test of Wilcoxon et al [31].

Results

A total of 46 strains of pneumococci could be typed. Table I shows the relations between clinical diagnosis and the various materials examined, with blood cultures, CSF and pleural fluids accounting for the majority. The diagnoses were in order of frequency: pneumonia (2), meningitis (14), primary peritonitis (4), otitis media (2), lymphadenitis (2), osteomyelitis (2), bacteraemia (1), and conjunctivitis (1). The seasonal distribution of the diseases is shown in Fig 1. From the data presented in this Figure it is apparent that the incidence showed a marked increase in the period from January to April and was less pronounced in the period between August and October. As far as pulmonary infections are concerned, the month of July showed the same morbidity rate as the month of April. For children with pneumonia, sex distribution showed a male to female ratio of 1.5:1. For children with meningitis the ratio was 1:1. For all the diseased children the ratio was in the order of 1:1.2. The median values for the age at which children had become ill were 37 months for children with pneumonia, 15 months for children with meningitis, and 24 months for all of the diseased children.

The age at which children fell ill ranged from 3/12 to 14 9/12 years. There was a significant difference in age distribution between children with pneumonia and those affected with meningitis ($\alpha = 0.05$). Of the children with pneumonia, six were two years of age or less (of a total of twenty), of those affected with meningitis, ten were two years of age or less (of a total of fourteen). And of all the diseased children, twenty-three were two years of age or less (of a total of forty-six).

Ten different serotypes were observed in our material. Serotypes of

Table I

Relationship between clinical diagnosis and kind of material examined (with presence of pneumococci detected)

Material examined	Clinical diagnosis					
	Pneumonia (N = 20)	Meningitis (N = 14)	Peritonitis (N = 4)	Osteomyelitis/ arthritis (N = 2)	Other diseases* (N = 6)	Total (N = 46)
Blood culture	9	3		1	2	15
CSF		11				11
Pleural fluid	11					11
Ear swab					2	2
Eye swab					1	1
Abscess aspirate					1	1
Bone aspirate				1		1
Peritoneal fluid			4			4

* = Lymphadenitis, otitis media, conjunctivitis, bacteraemia

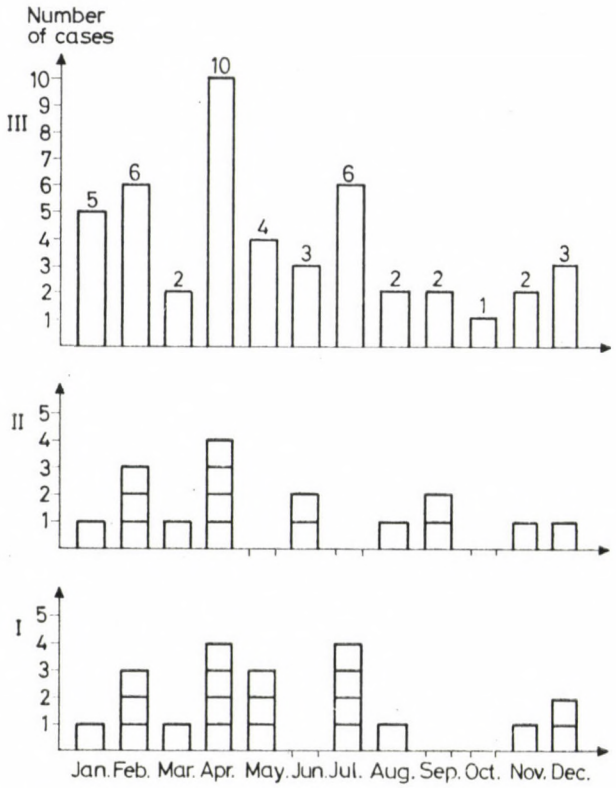

FIG. 1. Seasonal distribution of 46 pneumococcal infections. I = pneumonia, II = meningitis, III = all patients studied

pneumococci are shown in Fig 2. Table II shows a breakdown of serotypes according to the clinical diagnosis. Table III shows the distribution of serotypes in relation to age. Type 6 took the first place in the group of children aged 3 months to 2 years, it was not present in children aged 2 years to 4 years, and it appeared once only in patients over 4 years of age.

Type 1 was dominant in the latter group of patients; it took the first place in children aged 2 years to 4 years, and was not present in the group of youngest patients. Whereas type 14 was strongly represented in the two lower age groups, it was absent in the children over 4 years. The widest spectrum of isolated serotypes was found in the children aged 3 months to 2 years.

Of the 46 strains of pneumococci one strain showed, in the agar diffusion test, only a 12 mm zone of inhibition when penicillin G was used in a test dose of 3 I.U. Checking yielded the following minimum inhibitory concentrations: penicillin G, 0.125 μg/ml; ampicillin, 0.032 μg/ml;

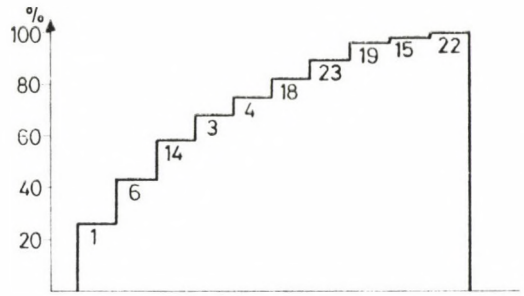

FIG. 2. Cumulative percentages of pneumococcal serotypes cultured from 46 children between April, 1980, and January, 1984

TABLE II

Frequency of occurrence of serotypes of pneumococci in various diseases (N = 46) during childhood beyond the neonatal period

Serotype	Pneumonia (N = 20)	Meningitis (N = 14)	Peritonitis (N = 4)	Osteomyelitis/ arthritis (N = 2)	Other diseases* (N = 6)	Total (N = 46)
1°	7	1	4			12
3°	4				1	5
4°	1	1			1	3
6°	3	3		1	1	8
14°	3	2			2	7
15		1				1
18°		3				3
19°	1	1		1		3
22		1				1
23°	1	1			1	3

* = Lymphadenitis, otitis media, conjunctivitis, bacteraemia
° = Serotypes which are, either directly or in form of subtypes, represented in a 14-valent vaccine (Pneumovax®)

TABLE III

Frequency distribution of serotypes of pneumococci in relation to age

Rank order	Patients							
	Total number (N = 46)		Three months to <2 years (N = 23)		Two years to <4 years (N = 11)		>4 years (N = 12)	
	Type	(Number of strains)	Type	(Number of strains)	Type	(Number of strains)	Type	(Number of strains)
1	1	(12)	6	(7)	1	(3)	1	(9)
2	6	(8)	14	(4)	14	(3)	6	(1)
3	14	(7)	19	(3)	3	(2)	3	(1)
4	3	(5)	3	(2)	4	(1)	22	(1)
5	4	(3)	4	(2)	18	(1)		
6	18	(3)	18	(2)	19	(1)		
7	23	(3)	23	(2)				
8	19	(3)	15	(1)				
9	15	(1)						
10	22	(1)						

oxacillin, 1.00 μg/ml. Evidence for beta-lactamase production was not obtained. The strain had been isolated from a child with otitis media and mastoiditis, and proved to be serotype 4. The child, before admission to our hospital, had been treated for an extended period with oxacillin in another hospital.

Discussion

A detailed description of the history of pneumococcal research was presented by Austrian [4]. The fact that pneumococci isolated from normal carriers and diseased subjects show polysaccharide capsules soon proved of particular interest. The different capsules provide the basis for classification into serotypes of which the total number is at present eighty-three (3).

Pneumococci that are merely present on the mucous membranes show, in so far as the frequency distribution of serotypes is concerned, a different pattern than those isolated in the case of invasive infections [24.] Acquisition of antibodies without clinical infections by pneumococci is appropriately explained by stimulation through homologous antigens (i.e. pneumococci carried in the nasopharynx) and cross-reacting antigens of other bacteria found on various body surfaces, e.g., Klebsiella spp., E. coli and some types of streptococci [16]. Results of both clinical and experimental studies provided ample evidence for the central position occupied by the spleen in the defense against pneumococcal infections [7, 15]. This is considered to explain the special disposition to infections by pneumococci of morphologically or functionally asplenic patients [7, 27]. Further dispositional factors for pneumococcal infections in infancy [surveys of which are given in 3, 7, 27] are: anatomical defects in the cranial region, sickle cell anaemia, malignancies (particularly Hodgkin disease), primary or secondary immunodeficiencies.

In cases of childhood pneumonia, pneumococci have been reported to be the most frequent causal agents [27]. As agents causing bacterial meningitis of childhood they generally are second or third in frequency [20, 27]. During childhood, infections by pneumococci occur above all in the first two years of life [22, 28]. As far as the principal types of manifestation during childhood are concerned, children with meningitis seem to be of the lowest average age.

From a detailed consideration of the observations analysed here it is reasonable to assume that, in the period under review, the diagnosis of pneumococcal pneumonia could be established just too infrequently. Detection of the presence of pneumococci in blood cultures and pleural fluids, respectively, reflects only part of the diseases since not all cases of pneumococcal pneumonia showed a simultaneous occurrence of bacteraemia and/or pleural involvement and because suitable material was not or could not be obtained in each case or a microbiological diagnosis was not made

until after the administration of antibiotic agents [3].

In our hospital, too, pneumococci take the third place as meningitis causing agents. As to the age at which the patient had fallen ill, our observations are in agreement with those reported in the literature. We, too, were able to find that the average age of children with meningitis, which is fifteen months, is markedly lower than that of children affected with pneumococcal pneumonia.

In the literature available to us, authors reported a more or less pronounced predominance of the male sex [27]. In our children such a predominance was shown only by pneumococcal pneumonia, whereas children with meningitis showed a substantially balanced ratio. The predominance of girls in the overall result might be considered as being due chiefly to the proportion of cases of primary peritonitis, a disease which occurs almost exclusively in girls during childhood.

In studies on the seasonal distribution of diseases produced by pneumococci, some authors observed a substantial increase in months with lower temperatures [22, 27]. Our results only showed a less frequent occurrence of pneumococcal infections between the months of August and October.

As a rule, determination of the serotypes of isolated strains of pneumococci was dispensed since the advent of antimicrobial chemotherapy. Interest in serotyping again arose in the last fifteen to twenty years. Reasons therefore can be summarized as follows [9, 19, 27, 32].

- Pneumococci continue to be causal agents of common and occasionally serious infections.
- Their importance for old and disposed patients is on the increase.
- There are not only penicillin G-resistant, but multiple resistant pneumococci.
- Production and controlled application of vaccines for the prevention of pneumococcal infections is desirable and feasible.
- There are indications of varying frequency distributions of major serotypes which differ in different geographic regions and, possibly, over larger periods of time and therefore require programmes of continuous surveillance to be instituted.

From the large number of previous studies on the frequency of occurrence of different serotypes in invasive infections it is evident that only about twenty types or subtypes can be regarded as being of special importance [6, 8, 9, 11, 12, 28, 29, 33]. They give the following series according to the frequency of occurrence:

Types 6, 14, 19, 23, 9, 15, 17, 18 [Gray et al, 23]

Types 14, 19, 6, 18 [Broome and Facklam, 8]

Types 19, 14, 23, 18, 4 [Weisholtz et al, 36]

Types 19, 6, 23, 14, 3, 18, 4, 9, 7, 1 [Gray et al, 22].

Special importance has been attached to type 14 in childhood [8, 23];

in our material, too, type 14 was frequent in the lower age groups, whereas it could not be found beyond four years of age. Type 1 is the most common with pneumonia and it is the only type that was identified in all of the cases of primary peritonitis. As regards pneumonia, in some reports [33] type 1 took the first place. Our observation of the frequent occurrence of type 6 in children under two years of age as compared with older children has been found also by other authors [22, 27].

On the basis of a large number of studies of the prevalence of serotypes of pneumococci responsible for invasive infections, some immunoprophylactic vaccines were developed containing the polysaccharides of the most important types (surveys are given in 3, 5). For example, there is a clinically tested and certified 8-valent [32], a 14-valent [1, 10], a 17-valent [18], and more recently a 23-valent [5, 28] vaccine. Of these, the 14-valent vaccine (Pneumovax®, MSD) found widest application in the USA [1] and later in other countries [3, 26, 35]. It contains the polysaccharide substance of types or subtypes 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F and 25 and, according to the studies cited, it is capable of covering effectively some 70 to 80% of all serious pneumococcal infections occurring in North America and Europe, since these are caused by corresponding or closely related serotypes. The serotypes found in diseased children correspond better to those contained in the vaccine than to the serotypes isolated from adults [22, 27, 28, 33]. Also, among the 46 strains of pneumococci which were isolated by us there are only two (namely, types 15 and 22 occurring once each, both isolated from children with meningitis) that did not correspond nor were related to any of the types contained in the vaccine. This means a level of agreement of approximately 96%.

As to the age of children, opinions are divided in certain points [for surveys 7, 21, 26, 28, 30, 32]. The most highly endangered age group, children up to two years of age, cannot probably be considered to come into question for immunization. In the case of splenectomy, it would be necessary that the inoculation be performed some four to eight weeks prior to the proposed surgical intervention; otherwise, vaccination would have to be performed at the earliest one or two weeks after the intervention.

Children with Hodgkin disease should, if possible, be vaccinated before therapy is started. Since a protective effect could not convincingly be demonstrated for the majority of indications, some authors [21, 30] recommend that immunization after splenectomy should be combined with antibiotic (e.g. penicillin) prophylaxis for three to five years and that these children be examined by the attending physician whenever they have a febrile disease.

It is of crucial importance that the resistance of pneumococci to antibiotics, and more particularly to penicillin, be most carefully controlled. Obviously, there are marked differ-

ences in resistance in different geographic regions [2, 13, 25, 34, 39]. In Europe, the situation seems to be favourable [12, 35, 38] although some papers [10, 14, 17] reported the occurrence of resistance or of reduced susceptibility. Of the 46 strains isolated by us only one proved to be of reduced susceptibility to penicillin G; in a child that had received oxacillin for a prolonged period of time.

Summing up, the following conclusions have been drawn.

- The distribution of serotypes of the strains of pneumococci isolated from different age and different disease groups should be surveyed on a broad basis.
- The resistance to antibiotic agents proposed to be used for therapeutical purposes should carefully be determined in every case, it being advisable for the results obtained to be included in a Chemotherapeutic Resistance Surveillance Program.
- The group of subjects up to two years of age should be included in a program undertaken with a view to arriving at a better understanding of the immune response to polysaccharide antigens and enabling new approaches to be adopted prior to inclusion in immunoprophylaxis of this particularly endangered group of patients.

ACKNOWLEDGEMENT

We are indebted to the Institute of Medical Microbiology and Epidemiology at Leipzig, for assistance in freeze-drying, and to Dr. W Witte, Wernigerode Institute of Experimental Epidemiology, for detailed studies.

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Received February 7, 1985

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