Archives of Disease in Childhood, 1986, **61**, 377–381

Carriage of penicillin resistant pneumococci

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SUMMARY A survey of 303 urban and 156 rural children showed nasopharyngeal carriage of relatively resistant *Streptococcus pneumoniae* organisms in 14·2% and 19·2% of children, respectively. These organisms have minimum inhibitory concentrations for penicillin in the range of 0·12–1 μg/ml.

An analysis of 40 relatively resistant *S. pneumoniae* strains showed resistance to co-trimoxazole in 47·5%, trimethoprim in 42·5%, fusidic acid in 20%, tetracycline in 2·5%, and rifampicin in 5%.

All the strains were susceptible to chloramphenicol and vancomycin, while the minimum inhibitory concentrations of third generation cephalosporins and imipenem were comparable with or lower than those of penicillin. Eighty three per cent of the strains tested belonged to serogroups 6 and 19. These findings are discussed in relation to the poor clinical response to treatment with penicillin for relatively resistant *S. pneumoniae* meningitis, and the minimum inhibitory concentrations of alternate agents under review for treatment of systemic pneumococcal disease are presented.

Penicillin G has been the drug of choice for the treatment of infections due to *Streptococcus pneumoniae* for many years. The isolation of a pneumococcus showing increased resistance to penicillin[1] and the subsequent isolation of resistant strains in the community[2] raised the possibility of widespread emergence of these strains and of therapeutic failures with penicillin in the treatment of pneumococcal infections. The possibility of such a prospect was, however, considered to be extremely remote,[3] especially in view of the isolated populations in which the resistant organisms occurred and widespread penicillin prophylaxis given to the community concerned.[2] Over the past decade, however, these organisms have been isolated from patients in many parts of the world and failures of treatment using penicillin have been documented, especially in pneumococcal meningitis due to relatively resistant *S. pneumoniae* organisms (0·12 < minimum inhibitory concentration < 1 μg/ml).[4–11]

In studies in Johannesburg the rate of nasopharyngeal carriage of antibiotic resistant pneumococci was shown to be high in paediatric wards where nosocomially acquired infections due to resistant strains occurred.[12] The nasopharynx as a source of pneumococci has obvious predictive potential[13] for the emergence of resistance in clinically significant isolates and has therefore been used to assess pneumococcal antibiotic resistance in different population groups.[14–17]

Screening of nasopharyngeal isolates has recently identified multiply resistant pneumococci in New York associated with clinical disease in three patients.[17] We now present data on the nasopharyngeal carriage of relatively resistant *S. pneumoniae* in children from geographically distinct rural and urban regions. In addition, we report the minimum inhibitory concentrations of 40 such strains to 15 additional antimicrobial agents, including several candidates for the treatment of meningitis due to these organisms.

Materials and methods

Isolation and identification of pneumococci. Calcium alginate swabs (Calgiswab type 1, Spectrum, USA) were used to collect the nasopharyngeal specimens. Pernasal swabs were immediately plated onto blood agar plates (Oxoid Columbia base with 5% horse blood) containing 5 μg/ml gentamicin.[12]

All plates were incubated overnight at 37°C in 5% carbon dioxide, and pneumococci were identified by

their typical colonial appearance. Confirmatory tests included optochin sensitivity, bile solubility, and capsular typing with antipneumococcal serum (Staten Seruminstitut, Copenhagen).

Antibiotic susceptibility testing. Antibiotic susceptibility was assessed by the disk diffusion method on 5% horse blood agar plates (Oxoid Columbia base). An inoculum of roughly 10^6 organisms was obtained by incubating five colonies in serum broth to a desired turbidity comparable with a barium sulfate standard (NCCLS Publications No ASM–2, 1975). Plates were inoculated with a sterile cotton swab on a wooden applicator and incubated aerobically at 37°C overnight. The disks used formed part of a Mastring–S (Mast Laboratories Ltd, UK) and included methicillin 5 μg, chloramphenicol 30 μg, tetracycline 30 μg, erythromycin 15 μg, clindamycin 2 μg, and rifampin 5 μg. Inhibition zone diameters around 5 μg methicillin disks were used to assess susceptibility of the pneumococci to penicillin as zone diameters around these disks correlate more accurately with penicillin G minimum inhibitory concentrations than the use of 6 μg penicillin disks.[12]

Strains showing resistance to methicillin on the disk diffusion plates were tested by the dilution technique to obtain minimal inhibitory concentrations. Dilutions of 15 antimicrobial agents as well as of penicillin were prepared and incorporated into Columbia oxoid agar plates containing 10% horse blood (except the trimethoprim and co-trimoxazole plates, which contained 5% lysed horse blood). A final inoculum of 10^4 colony forming units in 0·01 ml was applied to all of the plates using a Steers multipoint inoculator. The suspension of organisms was prepared from 10% serum broth. Minimum inhibitory concentration end points were determined after 18 hours incubation at 37°C.

Surveillance methods

Nasopharyngeal swabs were taken from all children aged under 5 years and the adult staff of three day care centres and a children's orphan home in Soweto. Similarly, nasopharyngeal swabs were taken from children aged under 5 from four rural villages in the north eastern Transvaal area of South Africa. The villages were isolated from each other and were all at least 30 kilometres from the nearest urban area. The children from whom nasopharyngeal swabs were taken in each village were all those living in a geographical area chosen by randomised cluster analysis.

Results

Table 1 illustrates the distribution of relatively resistant *S. pneumoniae* isolates in both urban and rural communities. The carriage rate of 14·2% in urban children means that 20·9% of pneumococci isolated showed intermediate resistance to penicillin. In the rural communities 30·2% of pneumococci isolated were relatively resistant *S. pneumoniae* strains. Five carriers were identified from 30 staff members (16·7%) in the urban centres, but no resistant isolates were found. Table 2 illustrates the minimum inhibitory concentrations of 15 additional antimicrobial agents for 40 relatively resistant *S. pneumoniae* isolates. Resistance to treatment with tetracycline (minimum inhibitory concentration >64 μg/ml) was noted in one isolate. Nineteen isolates showed resistance to treatment with co-

Table 1 *Nasopharyngeal isolates of pneumococci showing intermediate penicillin resistance*

	No of children	*No (%) of pneumococcal isolates*	*No (%) of isolates with penicillin minimum inhibitory concentrations >0·12 μg/ml*
Urban communities:			
Monde	104	73 (70·2)	13 (12·5)
Davenport	90	55 (61·1)	8 (8·9)
Othandweni	32	26 (81·2)	10 (31·2)
Silgee	77	52 (67·5)	12 (15·6)
Total	303	206 (68·0)	43 (14·2)
Rural communities:			
Mohlabe	28	20 (71·4)	8 (28·6)
Dan	31	23 (74·2)	11 (35·5)
Nwanitswaskop	50	30 (60·0)	7 (14·0)
Nwanitswaskraal	47	23 (48·9)	4 (8·5)
Total	156	96 (61·5)	30 (19·2)

Table 2 *In vitro susceptibility of 40 nasopharyngeal isolates of* Streptococcus pneumoniae *showing intermediate penicillin resistance*

Antimicrobial agents	*No of isolates inhibited by a minimum inhibitory concentration (MIC) (μg/ml) of*																			*MIC$_{50}$*	*MIC$_{90}$*
	<0·015	*0·015*	*0·03*	*0·06*	*0·12*	*0·25*	*0·5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*	*500*	*1000*	*2000*	*> 2000*	
Penicillin					16	11	11	2													0·16	0·45
Tetracycline					4	8	21	5	1					1							0·34	0·80
Erythromycin			40*																		<0·06	<0·06
Clindamycin			39*		1																<0·06	<0·06
Co-trimoxazole§										4	8	9	10	7	2						15	55
Trimethoprim						2	6	8	7	12	3	2									1·55	5·35
Imipenem	26	6	6	2																	<0·015	0·025
Ceftriaxone	2		2	5	13	16	2														0·11	0·23
Cefuroxime		5†		2	4	15	13	1													0·20	0·44
Cefotaxime	2		5	3	19	11															0·09	0·20
Ceftazidime		5†			1	2	1	7	14	10											1·3	3·2
Chloramphenicol								6	23	11											1·6	3·3
Vancomycin						5	24	11													0·41	0·83
Rifampicin			13*	4	18	1				2				1	1‡						0·07	0·25
Fusidic acid									3	7	11	11	8								7·6	24
Streptomycin																	5	17	16	2	950	1875

*Minimum inhibitory concentration <0·03 μg/ml.
†Minimum inhibitory concentration <0·015 μg/ml.
‡Minimum inhibitory concentration >128 μg/ml.
§Co-trimoxazole was constituted by a mixture of trimethoprim and sulfamethoxazole in a ratio of 1:19.

trimoxazole (minimum inhibitory concentration >16 μg/ml), while 17 isolates showed resistance to treatment with trimethoprim alone (minimum inhibitory concentration >2 μg/ml). Eight strains were resistant to treatment with fusidic acid (minimum inhibitory concentration >16 μg/ml) and two strains showed rifampin minimum inhibitory concentrations of >64 μg/ml. Finally, high level resistance to treatment with streptomycin was encountered in all relatively resistant *S. pneumoniae* isolates.

The lowest minimum inhibitory concentrations were found for imipenem. Of the third generation cephalosporins tested, ceftriaxone, cefuroxime, and cefotaxime had minimum inhibitory concentrations similar to that of penicillin. No isolates were resistant to treatment with chloramphenicol or to vancomycin.

The serotypes of 35 relatively resistant *S. pneumoniae* strains were type 4 (11%); type 6B (43%); type 14 (3%); type 19A (11%); type 19F (29%); and type 23B (3%).

Discussion

It has been known for many years that pneumococci, grown in the presence of subinhibitory concentrations of penicillin, will develop resistance in vitro to that antibiotic.[18] The first isolation of pneumococci resistant to treatment with penicillin in man was, however, only made in 1967.[1] In 1971 pneumococci were isolated from nasopharyngeal swabs on 15 New Guineans exposed to penicillin prophylaxis as part of a trial to reduce the mortality from pneumonia in that community.[2] Since that time pneumococcal resistance to treatment with penicillin has been identified as a problem in many hospitals,[14–16 19] and, indeed, multiple antibiotic resistance (including penicillin minimum inhibitory concentrations up to 4 μg/ml) were described in nosocomial outbreaks in South Africa in 1978.[12]

Our findings show very high rates of carriage of relatively resistant *S. pneumoniae* in black children. These observations give cause for concern given that there was no demonstrable antibiotic pressure for selection of resistant mutants in the communities and penicillin is only obtainable on prescription in South Africa. Indeed, the exposure to antibiotics in a rural community is low. The rate of carriage of resistant strains will probably vary with time as new pneumococcal strains are acquired or lost. The average duration of carriage of serotype 14 and serogroups 6, 19, and 23 in children has been shown to be roughly four months.[20] In support of these data we were only able to identify one carrier of

relatively resistant *S. pneumoniae* out of 11 retested six months after the initial study in the rural children (unpublished observations).

Carriage of pneumococci has been correlated with the emergence of clinical disease[13] to the extent that roughly one in six children colonised by new strains of pneumococci will develop disease due to that strain.[20] The emergence of a multiply resistant pneumococcus causing meningitis in an infant was linked to the nasopharyngeal carriage of similar organisms by children at the infant's day care centre.[21] The relatively resistant *S. pneumoniae* serotypes isolated in this study are responsible for the majority of serious pneumococcal diseases in children. Pneumococci of serogroups 6 and 19 together with serotype 14 are responsible for 28–57% of pneumococcal bacteraemias in children.[22–26]

It is of concern that infections caused by relatively resistant *S. pneumoniae* strains have been associated with a poor response to treatment with penicillin, especially in meningitis.[4–11] These reports suggested that penicillin concentrations in the cerebrospinal fluid (CSF) may be inadequate to cover infections with organisms in the minimum inhibitory concentration range of 0·1–1·0 μg/ml in the CSF during adequate treatment for meningitis. As a result of this, attempts have been made to find alternate therapeutic approaches to pneumococcal meningitis.[27 28] Third generation cephalosporins such as cefotaxime and ceftriaxone have been shown to have achievable CSF concentrations in excess of 5 μg/ml in experimental meningitis.[29]

The minimum inhibitory concentrations of imipenem are below the concentrations of this drug attainable in the CSF of patients with bacterial meningitis, at least in the presence of the renal dipeptidase inhibitor, cilastin (J Modaï, D Vittecoq, M Wolff, and A Meulemans. Abstract. 14th International Congress of Chemotherapy 1985; S–78–5: 253).

Our data suggest that these new antimicrobial agents may be considered as alternatives to chloramphenicol and vancomycin in the treatment of meningitis caused by relatively resistant *S. pneumoniae*.

The mechanism of resistance to treatment with penicillin in pneumococci is thought to be changes in penicillin binding proteins, which can be selected by incubation of the organisms in subinhibitory penicillin concentrations.[30] Production of β-lactamase does not seem to play a role in the development of resistance.[30 31]

Our findings of widespread relatively resistant *S. pneumoniae* in two discreet communities emphasises the importance of monitoring nasopharyngeal carriage of pneumococci and the urgent need for reconsideration of antimicrobial strategies against the pneumococcus.

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Received 7 January 1986

Commentary

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We have known for many years that antibiotic resistance can emerge not only in the test tube but also in patients and that it is important for clinical medicine. *Mycobacterium tuberculosis* is a good example, and, indeed, for years regimes have been chosen not only because they are effective in treating the disease but also because they minimise the occurrence of drug resistant microbes. Recently, penicillinase producing gonococci have arrived from abroad to plague us and again have forced changes in treatment schedules.

It is therefore important that we take seriously reports that penicillin resistant pneumococci are circulating and causing disease. This was well documented in Papua New Guinea and in mine workers in South Africa. Now we have a similar account of such organisms circulating among children in South Africa. Pneumococci are carried in the respiratory tract (and the rates are usually much higher in these countries than they are in the United Kingdom) and penicillin is widely used to treat infections, particularly of the respiratory tract. Thus it seems that over the years organisms have been selected with increased resistance to the drug. Although drug resistant mutants of bacteria may be of reduced pathogenicity, unfortunately, at least some of these pneumococci are apparently fully pathogenic.

Thus it seems there are two lessons to be learned. Firstly, that it is not true that *Streptococcus pneumoniae* is always fully sensitive to penicillin and will remain so. We should therefore adjust our use of the drug to reduce the selection pressure on the organism—that is, by prescribing it only when really needed and in appropriate doses. Secondly, in spite of all our efforts in this country resistant organisms may reach us here from elsewhere. If they do it will be necessary to assess their sensitivity to other drugs and plan new treatment regimes. If that occurs a careful study of this paper could be very helpful.