Pneumococci resistant to erythromycin

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Susceptibility to erythromycin was determined for all pneumococci isolated in one laboratory from clinical specimens between 1969 and 1977. All 4724 isolates examined prior to October 1973 were susceptible to erythromycin. From October 1973 to December 1977, 64 (0.71%) of 8995 pneumococcus isolates were resistant to erythromycin. The resistant strains were isolated from 38 patients living in six widely separated communities in Alberta.

The erythromycin-resistant strains were of nine capsular types, including six that often cause bacteremic disease and five for which resistance to erythromycin has not been reported hitherto.

Certain strains of type 33 and of type 15 were highly resistant, the minimum inhibitory concentration (MIC) of erythromycin being 2000 μg/mL; these strains were also highly resistant to lincomycin and clindamycin. Resistance in strains of other types was much lower, the MIC of erythromycin being 0.6 to 20 μg/mL, and all but one of these strains were susceptible to lincomycin and clindamycin. All the erythromycin-resistant pneumococci were susceptible to penicillin.

La sensibilité à l'érythromycine a été déterminée pour toutes les souches de pneumocoques isolées dans un laboratoire à partir de prélèvements cliniques recueillis entre 1969 et 1977. Les 4724 souches isolées examinées avant le mois d'octobre 1973 étaient toutes sensibles à l'érythromycine. Entre octobre 1973 et décembre 1977, 64 (0.71%) des 8995 souches de pneumocoques isolées étaient résistantes à l'érythromycine. Les souches résistantes provenaient de 38 patients habitant six communautés fort éloignées de l'Alberta.

Les souches résistantes à l'érythromycine étaient de neuf types capsulaires différents, et comprenaient six souches qui sont souvent la cause de bactérémie et cinq souches pour lesquelles une résistance à l'érythromycine n'avait pas été signalée jusque là.

Certaines souches de type 33 et de type 15 étaient fortement résistantes, la concentration minimum inhibitrice (CMI) de l'érythromycine étant de 2000 μg/mL; ces souches étaient aussi fortement résistantes à la lincomycine et à la clindamycine. La résistance des souches d'autres types était beaucoup plus faible, la CMI d'érythromycine étant de 0.6 à 20 μg/mL, et toutes ces souches sauf une étaient sensibles à la lincomycine et à la clindamycine. Tous les pneumocoques résistants à l'érythromycine étaient sensibles à la pénicilline.

Pneumococcal infections are no longer predictably responsive to penicillin therapy. Many strains of *Streptococcus pneumoniae* have been reported to be relatively resistant to penicillin,[1,2] and some strains with higher resistance to penicillin and other antibiotics have been found recently in South Africa.[3-5] These observations make increasingly important the detection of trends of emerging or increasing resistance of pneumococci to antibiotics commonly used as alternatives to penicillin, such as erythromycin.

Resistance to erythromycin in pneumococci from clinical specimens was reported in 1967,[6,7] but since then only a few resistant strains have been detected. We present in this paper information on the occurrence of erythromycin resistance in pneumococci isolated in Alberta from clinical specimens during the 9-year period 1969 to 1977, and an analysis of the resistant strains isolated.

Methods

Pneumococci were isolated from sputum and from swabs of the nose, throat, ear, eye, skin and wounds submitted for diagnostic purposes between 1969 and 1977 from patients in Alberta and the adjacent region of the Northwest Territories. Specimens were inoculated onto sheep-blood agar (Tryptose–blood agar base with yeast extract, Difco, Detroit) and the plates were incubated at 35°C either anaerobically (those of nose and throat swabs) or in air containing 10% carbon dioxide (all others). Identification of *S. pneumoniae* was confirmed by the organism's bile solubility and the inhibition of its growth on blood agar by an optochin disc (containing 5 μg of ethyl hydrocupreine hydrochloride). The capsular type was determined by the quellung test with sera from Statens Seruminstitut, Copenhagen.

Diffusion tests of bacterial susceptibility were performed on sheep-blood agar incubated in room air or in air containing 10% carbon dioxide when the strain otherwise did not grow. Discs containing 2 or 15 μg of erythromycin were used, and the inoculum was standardized by turbidity to contain approximately 10^8 colony-forming units. The minimum inhibitory concentration (MIC), taken as the minimum concentration of antibiotic with which there was no growth or fewer than 5 colonies, was determined by an agar-dilution technique. Mueller-Hinton agar plates containing 5% sheep blood and concentrations of erythromycin or other antibiotics in a twofold dilution series were inoculated with approximately 10^5 organisms by a replicator. The plates were read after 18 hours' incubation at 35°C in room air or in air containing 10% carbon dioxide.

All isolates of *S. pneumoniae* were tested with a 2-μg erythromycin disc. For those showing a zone of inhibition around the disc less than 14 mm in diameter the MIC of erythromycin was determined and a diffusion test was performed with a 15-μg erythromycin disc. *S. pneumoniae* was regarded as resistant to erythromycin when the MIC was 0.63 μg/mL or greater, which was eight or more times higher than that of susceptible control strains (with MICs of 0.08 μg/mL or less); the resistant strains were arbitrarily classified as having low, moderate or high resistance, as indicated in Table I.

Results

The relation between MIC and zones of inhibition around the 2-μg and 15-μg erythromycin discs is shown in Table I.

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Between January 1969 and September 1973 none of 4724 isolates of pneumococci showed resistance to erythromycin. In October 1973 the first erythromycin-resistant strain (with an MIC of 2.5 μg/mL) was detected; it was of type 14 and was isolated from a swab taken from a child's conjunctiva. From then until December 1977, 8995 isolates were tested and 64 (0.71%) were found to be resistant to erythromycin. The erythromycin-resistant strains were isolated from 38 patients, and only one strain from each patient is referred to hereafter. These patients lived in six communities (A to F) in Alberta, each separated from the others by 100 km or more. Details of the strains from the 38 patients are given in Table II.

Marked differences in the degree of resistance were detected among the 38 strains. Fourteen were highly resistant, with MICs of erythromycin of 2000 μg/mL; they were also highly resistant to lincomycin and clindamycin. Of the 14, 13 were found in community A (population 2000) in 1974 and 1975 but never subsequently, while the other strain, of distinct capsular type, was isolated in 1977 from a patient living hundreds of kilometres away.

Twenty-one strains of four capsular types had MICs of erythromycin of 2.5 to 5 μg/mL and were regarded as moderately resistant; with one exception, a type 14 strain, they were susceptible to lincomycin and clindamycin. One other strain, also regarded as moderately resistant, required the addition of 10% carbon dioxide to room air for growth, and in this atmosphere the MIC of erythromycin was 20 μg/mL (MIC for susceptible control strains 0.16 to 0.32 μg/mL); the strain was susceptible to lincomycin and clindamycin.

Two strains were noted to have low resistance to erythromycin, with MICs of 0.63 to 1.25 μg/mL.

All erythromycin-resistant pneumococci were susceptible to 0.02 μg/mL or less of penicillin, 0.32 μg/mL or less of tetracycline and 2.5 to 5 μg/mL of chloramphenicol.

The resistant strains were isolated from specimens taken from the upper respiratory tract, except in four instances — two specimens from ears and two from eyes. The patients were 3 months to 13 years old, but most were less than 2 years old. The clinical diagnoses included pneumonitis, bronchopneumonia, bronchitis, tonsillitis, otitis and conjunctivitis. It was not possible to determine whether the strains isolated were the cause of the clinical conditions being investigated. Five patients are known to have been receiving erythromycin therapy when the swab was taken; therapeutic information was unavailable for most of the other patients.

Table I—Relation for pneumococci between minimum inhibitory concentration (MIC) of erythromycin and zones of inhibition around discs 6 mm in diameter containing 2 or 15 μg of erythromycin*

Resistance to erythromycin	MIC (μg/mL)	Zone diameter (mm)	
		2-μg disc	15-μg disc
None	≤ 0.08	>22	26
Low	0.63	13	20
	1.25	11	18
Moderate	2.5	8 - 9	15 - 17
	5	8	12 - 14
	20†	8†	12 - 13†
High	2000	None	None

*Incubation was in room air for all strains except those dependent on carbon dioxide, which required incubation in air containing 10% carbon dioxide (indicated by daggers).

Discussion

Infections by erythromycin-resistant pneumococci were described in 1967.[6,7] Thereafter only a few single cases[8-10] and the detection of three strains in Poland[11] were reported until 1977, when multiply antibiotic-resistant strains, some of which were erythromycin-resistant, caused outbreaks of infection in South Africa.[3,5]

The search for erythromycin-resistant strains in Alberta, begun in

Table II—Details of erythromycin-resistant pneumococci isolated in Alberta between October 1973 and December 1977

Resistance to erythromycin	MIC (μg/mL)			Capsular type	No. of infected persons	Place(s) of residence	Year(s) of occurrence of infection
	Erythromycin	Lincomycin	Clindamycin				
High	2000	200 - 400	200 - 400	33	13	A	1974–75
				15	1	B	1977
Moderate	2.5 - 5	0.63 - 1.25	0.08 - 0.16	6	14	C,D	1976–77
				10	3	A	1975
				14	2	C	1975–76
				4	1	C	1977
	2.5	400	400	14	1	A	1973
	20*	2.5*	0.32*	9	1	E	1977
Low	0.63 - 1.25	0.08 - 1.25	0.04 - 0.16	8	1	F	1975
				23	1	A	1975
Sensitive control strains							
Incubated in room air	0.08	0.32 - 0.63	0.08 - 0.16				
Incubated in air containing 10% carbon dioxide	0.16 - 0.32	1.25 - 2.5	0.16				

*The MICs for this strain were determined in the presence of 10% carbon dioxide.

1969, was unproductive until October 1973; since then (until December 1977) 38 patients have been found to be infected with strains of differing resistance and various serologic type. A clear distinction was noted between highly resistant strains (with MICs of 2000 μg/mL, the highest resistance recorded so far) and those of lower yet substantial resistance (with MICs of 0.63 to 20 μg/mL). The multiply resistant South African strains, a cause of serious disease, included some that were resistant to erythromycin and clindamycin[3,5] and others that were susceptible.[4] The erythromycin-resistant strains found in Alberta were susceptible to penicillin, tetracycline and chloramphenicol, and only those that were highly resistant to erythromycin, and one of the moderately resistant strains, were also resistant to lincomycin and clindamycin. The strains resistant to lincomycin did not show the zonal pattern of resistance that has been observed with some strains of *S. pyogenes*.[12] Among the erythromycin-resistant pneumococci was a strain requiring carbon dioxide for growth; this is not uncommon.[13] Incubation in an atmosphere enriched with carbon dioxide, however, alters the MIC of certain antibiotics, including erythromycin,[14] so the results are not strictly comparable with those of isolates incubated in room air.

The data on place of residence and year of occurrence of infection by the resistant strains provide an example of a cluster of 13 patients infected with resistant strains of type 33 in a small town during a period of 14 months; resistant strains of this type were not detected subsequently in that area or elsewhere. We found no evidence of spread of resistant strains from one locality to another. It was not possible to obtain information about prior administration of erythromycin or other antibiotics to most of the patients, but five patients are known to have been treated with erythromycin before the resistant strain was isolated. In some previous reports of resistant strains erythromycin had been given to the patient beforehand,[6-8] but in others it had not.[10]

The strains we studied were mostly isolated from patients whose pneumococcal disease was not serious, and none was cultured from blood. Nevertheless, of the nine capsular types of erythromycin-resistant strains six (types 4, 6, 8, 9, 14 and 23) are constituents of a pneumococcal polysaccharide vaccine now licensed in Canada and the United States and prepared from the 14 types said to cause at least 80% of the bacteremic pneumococcal disease that occurs in the United States.[15] Likewise, of eight types reported to cause 52% of the pneumococcal pneumonia and 67% of the pneumococcal meningitis in Australia,[16] five were found among the erythromycin-resistant strains in Alberta. With our paper resistance to erythromycin in five types — 4, 8, 9, 10 and 14 — has been reported for the first time. Resistance has already been recorded in types 6,[7] 15,[6] 23[11] and 33[10] (type 70 in the American classification[17]). Previous reports also exist of erythromycin-resistance in types 3,[9] 11[8] and 19A[3,5] (type 57 in the American classification), for a published total of 12 types.

The significance of our findings is amplified by the fact that 2.4% of the pneumococci isolated between 1974 and 1976 in the same geographic region showed increased resistance to penicillin.[2] These strains were of types 6, 9, 10 and 19. No strain studied by us has been resistant to both penicillin and erythromycin, but in this laboratory in the last 4 years resistance to erythromycin or penicillin has been found in pneumococci of 7 of the 12 capsular types that cause 78% of the bacteremic pneumococcal disease in the United States.[18]

Erythromycin-resistant pneumococci are still uncommon, but are perhaps not as rare as the paucity of reports of their occurrence has suggested. The high resistance to erythromycin detected in two capsular types and to clindamycin in three types is disturbing. Assumptions of susceptibility of pneumococci to erythromycin and clindamycin are no longer justifiable in this part of Canada, and sensitivity tests should be performed whenever these antibiotics are considered for the treatment of severe pneumococcal disease.

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