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Haemophilus biotypes in respiratory disease

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Haemophilus influenzae may cause serious illness in childhood, particularly capsulated Pittman type b organisms.[1-3] In the respiratory tract of adults the organism is usually non-capsulated[4] and is frequently isolated during acute episodes or exacerbations of respiratory diseases.[5] We have observed that *H parainfluenzae* is clinically indistinguishable from non-capsulated *H influenzae*, suggesting that *H parainfluenzae* is pathogenic in the respiratory tract of adults.[6] Subdivision of *H influenzae* and *H parainfluenzae* into biotypes is now possible[7 8] and, although this has been performed in childhood illnesses,[2] our aim was to determine the epidemiological and clinical value of haemophilus biotyping in adult respiratory diseases.

Methods

Using the laboratory records for 1983 we undertook a retrospective study of the case notes of patients who had had *Haemophilus* species isolated from the respiratory tract. Sputum specimens were routinely inoculated on to horse blood agar and heated horse blood agar containing 10 units of bacitracin/ml and incubated for 18 hours at 37°C in 7% carbon dioxide. Specimens from patients with suspected anaerobic infections were incubated anaerobically for 48 hours. Non-haemolytic haemophilus organisms were identified by their morphological and colonial appearance and the demonstration of satellitism with *Staphylococcus aureus*. Differentiation between *H influenzae* and *H parainfluenzae* was then achieved by the ability of the organism to metabolise δ-amino laevulinic acid (HP+, HI−). *H influenzae* and *H parainfluenzae* were further subdivided into their biotypes by the method of Kilian.[7 8]

The following variables were recorded and analysed: (i) distribution of *H influenzae* and *H parainfluenzae* biotypes in different diagnostic groups; (ii) antibiotic sensitivities, determined by a standard disc susceptibility technique; (iii) frequency of isolation of multiple organisms; (iv) pus cell counts in undiluted sputum—samples being taken from the most purulent part of the specimen—were scored (1–10 cells/high power field (HPF) = 1, 11–20 cells/HPF = 2, 21 or more cells/HPF = 3).

Statistical analysis was performed with Student's t test and χ^2 tests.

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Results

A total of 1086 isolates of haemophilus were identified, successful subculture and biotyping being achieved in 574 of 765 *H influenzae* and 157 of 321 *H parainfluenzae* isolates. After exclusion of 105 isolates from general practice (showing the same biotype distribution as isolates from hospital) and repeat specimens, 574 separate clinical episodes were studied. The diagnostic groups were: chronic bronchitis (n = 201), asthma (n = 63), bronchial carcinoma (n = 107), bronchiectasis (n = 52), pneumonia (n = 38), and "others' (n = 113). In the last group 37% of patients had pre-existing pulmonary disease and 36% had undergone thoracic surgery. All diagnostic groups showed a similar biotype distribution (*H influenzae* I 15%, II 33%, III 17%, IV 6%, V 4%, VI 2%, VII <1%; *H parainfluenzae* I 6%, II 11%, III 6%). Recurring clinical episodes were not always attributable to the same biotype, though multiple specimens from a single clinical episode usually (but not invariably) had the same single biotype. Biotype had no bearing on the symptoms recorded in all diagnostic groups, and biotype distribution was similar both during and between exacerbations of chronic bronchitis (table).

Antibiotic resistance was not related to biotype and *H influenzae* and *H parainfluenzae* had similar percentages of resistant organisms: ampicillin 11% and 9% respectively; erythromycin 5% and 1·5%; tetracycline 15% and 21%; cotrimoxazole 1·5% and 0·8%; chloramphenicol 0·2% and 0·8%. In patients with bronchiectasis 30% of organisms were resistant to ampicillin.

Frequency of mixed growths of organisms was not correlated with biotype.

Mean sputum pus cell scores were lower for *H parainfluenzae* as a group (2·1) than for *H influenzae* (1·7) but biotype and underlying diagnosis had no obvious effect.

Discussion

Non-capsulated *H influenzae* is widely regarded as a pathogen but there is still controversy about the pathogenic role of *H parainfluenzae* in respiratory disease.[9] In this study and our previously reported work[6] we have shown that, despite lower sputum pus cell counts with *H parainfluenzae* than with *H influenzae*, the two organisms produce similar symptoms and give rise to similar incidences of mixed infections and antibiotic resistance, thus making them indistinguishable on clinical grounds.

We have found no association between haemophilus bio-

Biotypes isolated during and between exacerbations of chronic bronchitis (percentages in parentheses)

	Haemophilus influenzae								*Haemophilus parainfluenzae*			
Biotype	*I* n = 32	*II* n = 69	*III* n = 33	*IV* n = 6	*V* n = 9	*VI* n = 5	*VII* n = 1	*Total* n = 155	*I* n = 12	*II* n = 21	*III* n = 13	*Total* n = 46
Exacerbation	22 (22)	44 (44)	20 (20)	5 (5)	5 (5)	3 (3)	1 (1)	100	7 (24)	13 (45)	9 (31)	29
Non-exacerbation	10 (18)	25 (45)	13 (24)	1 (2)	4 (7)	2 (4)	0 (0)	55	5 (29)	8 (47)	4 (24)	17

type and either presenting symptoms or underlying disease category. These findings, and in particular the finding of a similar biotype distribution in chronic bronchitis both during exacerbations and when patients are clinically stable, reduce the possibility of developing a narrow spectrum vaccine to protect against exacerbations of chronic bronchitis or other acute respiratory illnesses due to haemophilus. The biotype distribution we have observed is similar to that seen in healthy children; and it seems likely that normally saprophytic non-capsulated strains may cause disease in adults with pre-existing disease of the bronchial tree—previous frequent exposure to antibiotics possibly altering the characteristics of the resident flora and facilitating this process. It has been suggested that in patients with chronic bronchitis acute exacerbations are caused by a change in haemophilus type, a stable organism being present between exacerbations but not necessarily recolonising after the exacerbation.[10] Although in this study almost all isolates from a single clinical episode were of the same biotype, this was not necessarily true of repeated episodes, lending some support to this theory. The present study was not designed to investigate this theory and a prospective study is now needed to define the role, if any, of biotyping of haemophilus in exploring the epidemiology of respiratory tract infections. From the present findings biotyping does not appear to contribute to the diagnosis, investigation, or treatment of adult respiratory infections caused by *Haemophilus* species.

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