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Respiratory tract infections due to *Branhamella catarrhalis*: epidemiological data from Western Australia

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SUMMARY

During a 3-year period *Branhamella catarrhalis* was isolated in significant numbers from 239 (1·3%) of 19488 specimens of sputum sent for routine microbiological examination at a 700-bed general hospital. The majority of patients (83%) were over 60 years of age and 65% were male. There was a distinct seasonal variation in isolations with a peak incidence during the winter and early spring, a pattern not found with other pathogens. Susceptibility to amoxycillin decreased by approximately 50% over the 3 years, corresponding to an increased incidence of beta-lactamase-producing strains. There were minimal changes in susceptibility to other antimicrobial agents. Underlying pulmonary disease was the major factor predisposing to *B. catarrhalis* infection, and 71% of patients were smokers or ex-smokers.

INTRODUCTION

In the past *Branhamella catarrhalis* has been considered a harmless commensal of the human upper respiratory tract. Now *B. catarrhalis* is increasingly recognized as an important cause of respiratory tract infection secondary to underlying pulmonary disease (Ninane, Joly & Kraytman, 1978; McLeod *et al.* 1983). Many recent isolates produce a beta-lactamase enzyme (Doern *et al.* 1980; Ahmad *et al.* 1984), and the ability of diagnostic laboratories to correctly identify *B. catarrhalis* has assumed some importance (Doern & Morse, 1980). Despite the emerging significance of *B. catarrhalis* as a respiratory tract pathogen little is known about the epidemiology of such infections.

The aim of this investigation was to study the occurrence of *B. catarrhalis* respiratory tract infections in adult patients attending a large general hospital. The relationship between *B. catarrhalis* and other respiratory pathogens was determined and the susceptibility of isolates to antimicrobial agents monitored over a 3-year period. The distribution by age and sex of patients with *B. catarrhalis* respiratory tract infections was analysed together with predisposing factors, and the possibility of a seasonal trend in infections assessed. Finally, as a background

to the study, the nasopharyngeal carriage rate for *B. catarrhalis* was determined in a healthy adult population.

MATERIALS AND METHODS

Nasopharyngeal carriage

Nasopharyngeal swabs were collected from 123 adults (20–60 years old) with plain sterile buffered cotton swabs. None had any evidence of respiratory tract illness at the time of sampling or had been receiving antimicrobial chemotherapy. The swabs were placed immediately into a selective enrichment broth comprised of heart infusion broth (Oxoid) supplemented with 0·3% yeast extract powder (Oxoid), and containing 5 mg/l vancomycin and 8 mg/l trimethoprim. After overnight incubation at 37 °C the broth cultures were inoculated on to a selective agar medium, similar to that described by Corkill & Makin (1982), consisting of GC agar base (Oxoid) supplemented with haematin (Gibco) and Isovitalex (BBL) and containing 5 mg/l vancomycin and 8 mg/l trimethoprim. After 48 h incubation at 37 °C in an atmosphere of 10% carbon dioxide in air, colonies of oxidase-positive Gram-negative cocci were subcultured for further identification.

Specimens of sputum

A total of 19488 sputa, submitted to the Combined Clinical Microbiology Service for routine microbiological examination from November 1982, until October 1985, was included in the study. The laboratory provides a diagnostic service for the Sir Charles Gairdner Hospital, a 700-bed general hospital with a large specialist respiratory medicine unit.

The macroscopic appearance of each specimen was noted. A purulent portion was removed with a sterile swab or wire loop and smeared over a glass slide. After Gram-staining the smear was examined microscopically and assessed for suitability for culturing using criteria similar to those of Murray & Washington (1975). Suitable specimens were processed using the quantitative dilution technique described by Wilson & Martin (1972) with 0·85% saline as diluent. Culture media used were MacConkey agar incubated aerobically, together with blood and chocolate agar incubated in 10% carbon dioxide in air. After overnight incubation at 37 °C, a significant bacterial growth was considered to be greater than 25 colonies on the dilution plates, corresponding to greater than 10^7 organisms per ml of sputum.

Presumptive colonies of *B. catarrhalis* were identified using the criteria of Doern & Morse (1980). Other organisms were identified using appropriate microscopic, biochemical and serological techniques.

Susceptibility testing

The susceptibility of *B. catarrhalis* to antimicrobial agents was determined using a disk diffusion technique with Isosensitest agar (Oxoid) containing 6% lysed horse blood. Agar plates were inoculated to give semi-confluent growth and the following disks (all obtained from Oxoid) added: amoxycillin 2 μg; tetracycline 30 μg; erythromycin 15 μg; trimethoprim 1·25 μg; sulphamethoxazole 25 μg; cotrimoxazole 25 μg. A susceptible strain of *Staphylococcus aureus* (NCTC 6571)

was used as the control. Beta-lactamase activity was determined using a chromogenic cephalosporin (Nitrocefin, Glaxo).

Assessment and clinical data

Before the isolation of *B. catarrhalis* was considered significant the following criteria required fulfilling: (1) direct microscopy showed numerous polymorphs with no or few epithelial cells, together with moderate to numerous intra- and extra-cellular Gram-negative cocci, and (2) greater than 10^7 *B. catarrhalis*/ml of sputum in the absence of any obvious oropharyngeal contamination.

For the purpose of this report mixed infections have been excluded as it may have been difficult to define the significance of *B. catarrhalis* when well-recognized pathogens such as *Haemophilus influenzae* and *Streptococcus pneumoniae* were also isolated. However, the presence of beta-lactamase-producing *B. catarrhalis* was always noted in keeping with recent evidence for indirect pathogenicity (Smith & Lockwood, 1986).

The medical records of 100 randomly selected patients from whom a significant growth of *B. catarrhalis* was obtained were examined and relevant information abstracted.

RESULTS

Nasopharyngeal carriage

From the 123 nasopharyngeal swabs, 143 oxidase-positive, Gram-negative cocci were isolated. Only three isolates proved to be *B. catarrhalis*, giving a carriage rate of 2·4% in healthy adults. The remaining isolates were identified as *Neisseria subflava*, *N. flavescens*, *N. sicca* and *N. meningitidis*.

B. catarrhalis *respiratory tract infections*

Recognized respiratory pathogens were isolated from 3340 (17·1%) of the 19488 sputa examined during the 3-year study. Infections with *B. catarrhalis* totalled 239 (1·3%) for the period, with yearly isolations of 71 (1·2%), 94 (1·5%) and 74 (1·2%). The average isolation rates for other respiratory pathogens during the same period were; *H. influenzae* 4·7%, *Pseudomonas aeruginosa* 2·8%, *Strep. pneumoniae* 2·0 %, *Staph. aureus* 1·5% and *Klebsiella pneumoniae* 1·3%. The relatively high incidence of infections with *Ps. aeruginosa* was due to the large number of patients with cystic fibrosis attending the specialist respiratory medicine unit.

Age- and sex-related incidence

Fig. 1 details the distribution by age and sex of patients with *B. catarrhalis* respiratory tract infections during the study period. Most patients were in the older age groups; 83% were over 60 years of age, but all age groups were represented. The most susceptible age group was 70–79 years and nearly 30% of patients came from this group.

Males formed 65% (155) of the 239 patients with *B. catarrhalis* infection. During the study the average ratio of male to female patients attending hospital was 1·1:1. In the 60–79 years age group there was a strikingly increased proportion of

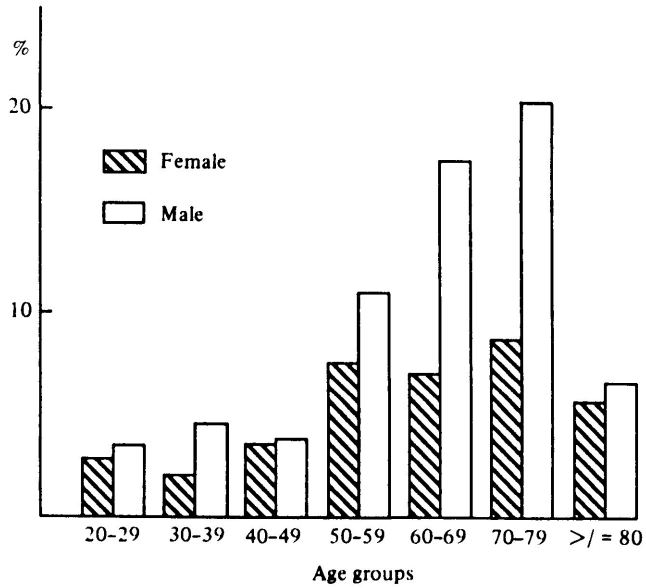

Fig. 1. Distribution by age and sex of patients with *B. catarrhalis* respiratory tract infections.

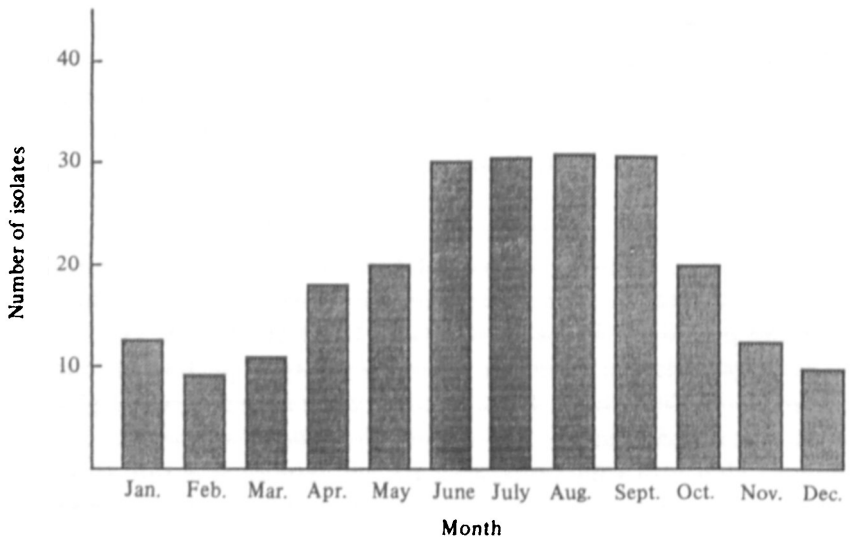

Fig. 2. Average monthly isolations of *B. catarrhalis* during the 3-year study.

males to females, compared to other age groups. The ratios of males to females in the various age groups were as follows: 20–29 years, 1·3 : 1; 30–39 years, 2·2 : 1; 40–49 years, 1·1 : 1; 50–59 years, 1·5 : 1; 60–69 years, 2·5 : 1; 70–79 years, 2·4 : 1; 80 years or more, 1·2 : 1. This finding was consistent for each of the three 12-month periods studied.

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Seasonal incidence

The 3-year study allowed an estimate to be made of possible seasonal variations (Fig. 2). During all three yearly periods there was a significantly lower incidence during the summer months. The only deviation from this trend came in January 1985, when an unexpectedly high number of isolations was recorded (7) when compared to January 1983 and 1984 (3 and 2 respectively). The reason for this could not be determined. The peak incidence occurred during the winter months. Overall these results were statistically significant when tested for seasonality using the method of Edwards (1961) (Chi squared = 47·028, $P < 0{\cdot}0001$). In order to determine whether the increased winter incidence of infection occurred only with *B. catarrhalis*, the monthly isolation rates for *H. influenzae* and *Strep. pneumoniae* were compared with those for *B. catarrhalis* for the calendar year 1984 (Fig. 3). Surprisingly, there were three distinct patterns; a winter peak incidence for *B. catarrhalis*, a summer peak incidence for *H. influenzae* but little variation in monthly isolation rate for *Strep. pneumoniae*.

Susceptibility to antimicrobial agents

Table 1 shows the susceptibility of *B. catarrhalis* to six antimicrobial agents for the six 6-month periods from November 1982 to October 1985. During this time susceptibility of strains to amoxycillin decreased from 64% to 31%. For the 6 months November 1984 to April 1985, resistance to amoxycillin increased to 90% associated with an unexpectedly high number of isolates in January 1985. Resistance was always associated with the production of a beta-lactamase enzyme. There were no significant changes in susceptibility to the other five antimicrobial agents for the same period. All strains were uniformly susceptible to tetracycline and resistant to trimethoprim. Susceptibility to erythromycin, sulphamethoxazole and cotrimoxazole varied slightly.

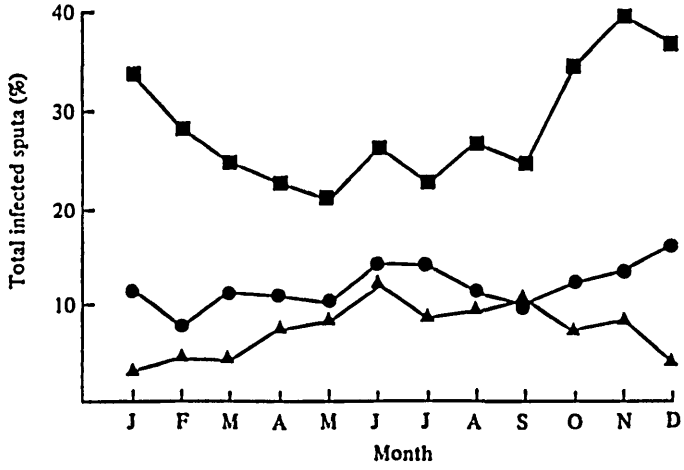

Fig. 3. Monthly isolation rates for *B. catarrhalis* (▲—▲), *H. influenzae* (■—■) and *Strep. pneumoniae* (●—●) during 1984.

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Table 1. *Susceptibility of* B. catarrhalis *to six antimicrobial agents during the 3-year study period*

	6-month periods					
	1*	2	3	4	5	6
Amoxycillin	64†	44	44	35	10	31
Erythromycin	93	86	100	97	95	93
Tetracycline	100	100	100	100	100	100
Sulphamethoxazole	96	86	100	97	100	96
Trimethoprim	0	0	0	0	0	0
Cotrimoxazole	96	86	100	97	100	96
Number tested	28	43	25	69	19	55

* 1, November 1982–April 1983; 2, May 1983–October 1983; 3, November 1983–April 1984; 4, May 1984–October 1984; 5, November 1984–April 1985; 6, May 1985-October 1985.
† Expressed as percentage susceptible.

Table 2. *Clinical details of patients with pulmonary disease*

Chronic obstructive airways disease/bronchitis	46
Bronchiectasis	6
Asthma	13
Lung carcinoma	9
Fibrosing alveolitis	1

Table 3. *Smoking of habits of 100 patients (63 males and 37 females) with* B. catarrhalis *respiratory tract infections*

Group	*n*	Amount/time	*n*
Smokers	37	> 15/day	30
		< 15/day	1
		Unknown	6
Ex-smokers	34	> 5 years	17
		< 5 years	14
		Unknown	3
Non-smokers	29	> 15/day	18
		< 15/day	5
		Unknown	11

Clinical data

Of the 100 randomly selected patients with *B. catarrhalis* respiratory tract infections, 63 were males and 37 were females (mean age 66 years, range 18–90 years), a ratio almost identical to the overall study. Clinical details of 62 of these patients with pulmonary disease are given in Table 2. Eleven patients had more than one complaint. Additional complicating factors in these patients were alcoholism in 13, oral corticosteroid therapy in 11, myocardial infarct in 3 and peripheral vascular disease in 3. In the 38 patients with no primary lung disease the major predisposing factors were carcinoma in 7, high risk of aspiration from the oropharynx in 7, myocardial infarct in 5, peripheral vascular disease in 4,

ischaemic heart disease in 4, diabetes in 3, alcoholism in 3 and super-infection in 3.

The smoking habits of the 100 patients are shown in Table 3; 26 of the 63 males were smokers, 26 were ex-smokers and 11 were non-smokers, while of the 37 females, 11 were smokers, 8 were ex-smokers and 18 were non-smokers.

DISCUSSION

The role of *B. catarrhalis* in pulmonary infection has received attention only in the last few years. Several recent reports (McLeod *et al.* 1983; Slevin, Aitken & Thornley, 1984) prompted a retrospective analysis of our institution's laboratory records for a 3-year period. Some interesting and, to our knowledge, previously undocumented trends became apparent.

The nasopharyngeal carriage rate of *B. catarrhalis* in healthy adults was 2·4%. Although many older texts state that *B. catarrhalis* (formerly *Neisseria catarrhalis*) is found commonly in the upper respiratory tract, there is little recent published information. Previously *B. catarrhalis* was isolated from 15% of pharyngeal swabs if a selective medium was used, while without a selective medium the isolation rate was 3% (Berger, 1961, cited by von Graevenitz & Rathbone, 1981). Schalen *et al.* (1980) failed to culture *B. catarrhalis* from any of 129 nasopharyngeal swabs from healthy individuals using non-selective media. Thus it may be concluded that nasopharyngeal carriage of *B. catarrhalis* in adults is not common. Carriage of a variety of *Neisseria* spp., however, is frequent; common isolates in the present study were *N. subflava*, *N. flavescens*, *N. sicca* and *N. meningitidis*.

It seems likely that the spuriously high carriage rate for *B. catarrhalis* derives from early misidentification of *Neisseria* spp. as *B. catarrhalis*. Many laboratories have traditionally relied only on the lack of fermentation of glucose, lactose, maltose and sucrose to identify these organisms. However, *N. flavescens*, for example, is impossible to distinguish from *B. catarrhalis* on this basis alone. Additional tests such as nitrate and nitrite reduction, tributyrin hydrolysis and utilization of 5% sucrose to form polysaccharide (Vedros, 1984) should be employed.

A retrospective survey similar to ours was reported by McLeod *et al.* (1983). Although it was difficult to calculate their yearly isolation rates, it was apparent that their isolation rate increased between 1981 and 1983. The only isolation rate that could be calculated from their data was for the 6 months November to April 1982–3, which included the winter months for the Northern hemisphere, when a figure of 2·6% was recorded. For May to October 1983, including the winter months in the Southern hemisphere, our isolation rate was 1·3%. Similar numbers of samples were examined during these two periods; 3071 in the study by McLeod *et al.* and 3365 in the present study. The higher isolation rate reported by McLeod *et al.* may be a reflection of the harsher winter conditions experienced in Edinburgh, Scotland, compared to the relatively mild conditions found in Perth, Western Australia.

Despite Perth's milder winter, we were able to show a statistically significant seasonal variation in the incidence of *B. catarrhalis* respiratory tract infections. Although it might be argued that there should be a general increase in respiratory

tract infections during the winter, this was clearly not the case (Fig. 3). The incidence of *H. influenzae* respiratory tract infections fell significantly during the winter months, while the incidence of infections with *Strep. pneumoniae* fluctuated little. McLeod *et al.* (1983) reported a similar seasonal variation in *B. catarrhalis* infections but failed to comment on the significance of their observation, while Smith & Lockwood (1986) suggested that there was little seasonal variation in a New Zealand hospital. Further studies are required to clarify this point. It may be that a preceding viral respiratory tract infection is important, but this remains to be demonstrated.

Most of our isolations of *B. catarrhalis* were from elderly patients, a similar pattern to that reported by McLeod *et al.* (1983) and Slevin *et al.* (1984), where the average age of patients was 67 years and 64·4 years respectively. Another important factor was the predominance of male patients. Sixty-five per cent of our patients were males confirming, the earlier findings of Slevin *et al.* (1984) (64%) and Smith & Lockwood (1986) (65%). In our study this was a real difference as there were approximately equal numbers of male and female patients in the hospital during the study period. Thus the greater susceptibility of male patients was a consistent finding in both Northern and Southern hemispheres. It is interesting to speculate on the reasons for this. One factor which correlated well was a history of smoking; over 82% of male patients were, or had been, smokers while the figure for females was 51%. Nevertheless there were a few previously healthy non-smoking adults with infection, supporting the suggesting of Slevin *et al.* (1984) and McLeod *et al.* (1986) that there has been an increase in virulence among strains of *B. catarrhalis*.

It is difficult to tell whether the increase in incidence of *B. catarrhalis* respiratory tract infections is real. There was little change in our isolation rate during the 3-year study period suggesting increased awareness, although comparable data from a period predating our study may be required to clarify this point. There was, however, a significant increase in the incidence of beta-lactamase-producing strains. The reason for this was not apparent but selective pressure through the use of ampicillin and amoxycillin may have played a part (McLeod *et al.* 1986). The rapid increase in resistance to penicillins has lead to the suggestion that beta-lactamase production may be plasmid-mediated (Kamme *et al.* 1986; Smith & Lockwood 1986). In our experience, and that of others (Ahmad *et al.* 1984), this property has always been chromosomally mediated, although a role for transposable elements may yet be found. Another interesting possibility is nosocomial spread of *B. catarrhalis* infection due to survival of strains in dried sputum for several weeks (McLeod *et al.* 1986). During our study there was circumstantial evidence for this in January 1985, when an unexpectedly high number of isolations occurred, the majority of which produced a beta-lactamase. Before this problem can be resolved a suitable typing scheme will be required.

Although some questions remain unanswered it is clear that *B. catarrhalis* is capable of causing severe, and sometimes fatal, infection. Not only may it be an important pathogen on its own but it may protect penicillin-susceptible strains of *H. influenzae* and *Strep. pneumoniae* through the production of a beta-lactamase and hence it should be reported in the presence of these organisms (Smith & Lockwood, 1986).

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