Comparison of genital and respiratory carriage of *Haemophilus parainfluenzae* in men

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Summary. In the first study, genital carriage of *Haemophilus parainfluenzae* was investigated in 103 women and 292 men attending a clinic for genitourinary medicine. In a second study, pairs of urethral and throat swabs were studied in 279 men. The vaginal carriage was 2%, urethral 12.4% and throat 13.3%. Biotype 2 was found to be a genital type and throat carriage of this biotype was significantly associated with its concomitant urethral carriage. Biotypes 1 and 3 were mainly found in the throat. Biotype 2 was significantly more likely to be resistant to ampicillin, tetracycline and sulphonamide but biotype 1 was significantly more likely to be resistant to trimethoprim.

Introduction

Haemophilus influenzae is a recognised respiratory pathogen and is also occasionally responsible for genitourinary infection. Albritton *et al.* (1982) reported that *H. influenzae* strains isolated from the genitourinary tract generally differed in biotype from respiratory isolates. Antibiotic-susceptible genitourinary strains contained plasmids more frequently than antibiotic-susceptible respiratory strains. *H. parainfluenzae* is generally regarded as a commensal in the respiratory tract and may cause sporadic local or systemic infections. Detailed studies of the distribution of biotypes in different body sites and their patterns of antibiotic sensitivity have not so far been carried out. We report the results of a study of respiratory and genital isolates of *H. parainfluenzae* in patients attending a clinic for genitourinary medicine.

Patients and methods

Study 1

The first study was performed from October 1983 to January 1984. A vaginal or urethral swab was taken from randomly chosen female (103) and male (292) patients attending the clinic for genitourinary medicine, St Stephen's Hospital, London. Swabs were inoculated immediately after collection on to 7% v/v heated horse-blood Columbia Agar (Oxoid) in halved plates, one half of which contained vancomycin 5 mg/L. The plates were incubated immediately for 2 days at 35°C in an atmosphere containing CO₂ 7%. Small gram-negative pleomorphic coccobacilli from a single colony were

examined for X- and V-factor dependency on Nutrient Agar (Oxoid). V-dependent isolates were then tested for the ability to metabolise α -aminolaevulinic acid. *H. parainfluenzae* strains thus identified were biotyped by the scheme of Kilian (1976).

Study 2

The second study was performed from October 1984 to March 1985. A pair of urethral and throat swabs was taken from each of 279 randomly chosen male clinic attenders. The paired swabs were used to inoculate each half of a heated blood-agar plate containing vancomycin 5 mg/L. The plates were incubated as described above. For each specimen, small gram-negative pleomorphic coccobacilli from three separate colonies were examined further. MicroScan HNID panels (American MicroScan, New Jersey) were used for the identification and biotyping of V-dependent isolates.

Antimicrobial susceptibility tests

Susceptibility to ampicillin, sulphonamide, chloramphenicol, tetracycline and trimethoprim was tested by disk diffusion on lysed blood DST (Oxoid) plates containing NAD 10 mg/L as described by Philpott-Howard and Williams (1982). The minimum inhibitory concentrations (MICs) of ampicillin, sulphonamide, chloramphenicol, tetracycline and trimethoprim were measured for those strains in the second study which showed a reduced zone diameter (<20 mm) with one or more of the antibiotics tested (Philpott-Howard and Williams, 1982). For isolates obtained in the first study, MICs of ampicillin only were measured. β -Lactamase production was tested with a chromogenic cephalosporin (O'Callaghan *et al.*, 1972). Chloramphenicol-resistant isolates were screened for the production of acetyltransferase by the method described by Manten *et al.*

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(1976). Isolates of the same biotype from the same site but with a more than fourfold difference in the MICs of any antibiotic tested were regarded as distinct from each other.

Results

In the first study with vaginal and urethral swabs from 103 female and 292 male patients, *H. parainfluenzae* and *H. influenzae* were each isolated from two vaginal swabs (2%). In the second study with pairs of urethral and throat swabs from 279 male patients, *H. parainfluenzae* was isolated from both the throat and the urethra of 17 men. The numbers and biotypes of *H. parainfluenzae* obtained from men in both studies are shown in table I.

Throat swabs from five patients and urethral swabs from three patients in the second study yielded more than one biotype of *H. parainfluenzae*. Over the periods of the two studies, a urethral swab

was obtained on more than one occasion from 50 men and a throat swab from 11. The 17 urethral isolates were obtained from 15 men; only two men had positive cultures on more than one occasion, 2 and 12 months between clinic visits respectively. Both had biotype 2. Of the 22 throat swabs, eight from seven men yielded *H. parainfluenzae*. Only one man had positive cultures on more than one occasion, with colonies of biotype 1 isolated from the first swab and of biotypes 1 and 3 from the second swab 2 months later.

Table II gives the pattern of antibiotic resistance of isolates obtained from the paired urethral and throat specimens. Lancaster and Irwin's method for partitioning χ^2 showed that biotype 2 was significantly more likely to be resistant to ampicillin ($p < 0.005$), tetracycline ($p < 0.01$) and sulphonamide ($p < 0.01$) than biotypes 1 and 3. Biotype 1 was significantly more likely to be resistant to trimethoprim than biotypes 2 and 3 ($p < 0.001$). The

Table I. The isolation and distribution of biotypes of *H. parainfluenzae* from the two studies

Study no.	Site	Number of patients		Number of <i>H. parainfluenzae</i> isolates of biotype					
		Total	<i>H. parainfluenzae</i> positive	Total	1	2	3	4	NT
1	U	292	37	37	2	32	3	0	0
2	U	279	34	51	0	46	3	1	1
				*(30)	(0)	(29)	(1)		
2	T	279	37	50	14	12	17	7	0
				(26)	(7)	(11)	(5)	(3)	

U = male urethra; T = male throat.

*( ) = Number of isolates from the 17 patients who had positive cultures from both sites.

Table II. Antibiotic susceptibility of *H. parainfluenzae* isolates obtained from the second study

Biotype	Number of isolates	Number of isolates with MIC (mg/L) of									
		ampicillin		chloramphenicol		trimethoprim		tetracycline		sulphonamide	
		<4	≥4	<4	≥4	<4	≥4	<4	≥4	<32	≥32
1	14(5)*	14	0	12	2	10	4	10	4	14	0
2	58(14)	46	12	49	9	56	2	29	29	47	11
			($p < 0.005$)						($p < 0.01$)		($p < 0.01$)
3	20(17)	20	0	20	0	20	0	17	3	20	0
4	8(6)	8	0	8	0	8	0	6	2	8	0

*( ) = number of isolates with a zone of inhibition ≥ 20 mm with all antibiotics tested; they are included in the figures under the sensitive range of MICs.

†p value calculated according to Lancaster and Irwin's method for partitioning χ^2 . Everipp (1977).

number of isolates of biotype 4 was too small to be included in the analysis.

β -Lactamase was produced by two isolates from the first and three from the second study. All five strains were of biotype 2 and had an MIC of ampicillin ≥ 32 mg/L. They were also resistant to chloramphenicol and tetracycline but sensitive to trimethoprim and, with one exception, also to sulphonamide. The six isolates, all of biotype 2, with MICs of chloramphenicol ≥ 16 mg/L, were shown to produce chloramphenicol acetyl-transferase.

Five multiresistant isolates were examined by the Plasmid Analysis Unit, Division of Hospital Infection, Central Public Health Laboratory, Colindale, London. Two isolates from the same patient contained no detectable plasmid. Another two isolates from different patients had identical plasmid profiles, containing at least three plasmids of 9.7, 3.6 and 1.5 kb. The fifth isolate also contained the 3.6-kb plasmid and possibly a 7.1-kb plasmid. None of the plasmids detected were capable of self-transfer.

Discussion

A study on the respiratory and genital carriage of *H. parainfluenzae* has not so far been reported. We studied randomly chosen patients in the sexually active group who attended the clinic for genitourinary medicine. Because of logistic difficulties, information about the presence or absence of genital symptoms or signs, microbiologically confirmed genital infection, and antibiotic therapy was not obtained for correlation in our survey.

In both studies, heated-blood-agar plates containing vancomycin 5 mg/L were used for the isolation of *H. parainfluenzae*. Although this medium allowed easy recognition of *Haemophilus* spp. in male urethral swabs, the presence of vancomycin-resistant commensals made such recognition difficult in swabs from other body sites. This may explain the apparently low vaginal carriage rate of *H. parainfluenzae* and *H. influenzae* (4% in our study and 0–2% in other reports—Albritton *et al.*, 1982; Messing *et al.*, 1983; Lamont *et al.*, 1986). Nevertheless, using heated-blood agar containing vancomycin, bacitracin, clindamycin and amphotericin B, Sturm (1986a) found 8% of vaginal secretions to contain one or other species of *Haemophilus*. These two *Haemophilus* spp. are probably not, therefore, commonly present in the vagina (<10%). Of the men we studied, 13% carried *H. parainfluenzae* in their throats. Chapin and Doern (1983) demonstrated much enhanced isolation of *H. influenzae*

from paediatric pharyngeal swabs by including vancomycin, clindamycin and bacitracin in the heated-blood-agar plates. Our results may, therefore, represent an underestimate of the carriage rate of *H. parainfluenzae* in the throat.

In the second study, three *Haemophilus* colonies randomly chosen from each positive plate were tested for susceptibility to a range of antibiotics, and biotyped. We found that 9% of men carried multiple biotypes in the urethra and 14% in the throat. Examination of a greater number of colonies plus the use of a more selective medium for respiratory isolation may yield a greater proportion of men carrying multiple biotypes. Only two of the 16 men were shown to be urethral carriers on more than one occasion. Whether this reflects transient or intermittent urethral carriage is not certain, since the possible effect of antibiotic therapy could not be excluded.

Several authors have reported the predominance of biotype 2 in male genital isolates (Messing *et al.*, 1983; Sturm, 1986a). The results of our study show conclusively that biotype 2 is a genital type. Over 88% (78 of 88) of urethral isolates belonged to this type and other biotypes were found only infrequently at this site. Of the 37 men with respiratory carriage of *H. parainfluenzae*, biotype 2 was isolated from nine, and eight of them also had concomitant urethral carriage of type 2. The occurrence of this type in the throat is therefore significantly associated with its urethral carriage ($p < 0.001$). This finding would suggest its orogenital transmission and its ability to survive in the throat; in contrast, the male urethra appears not to offer a favourable environment for other biotypes.

Our results showed that biotypes 1 and 3 are mainly respiratory types. Comparison of our results with those reported so far is difficult because of the difference in the types of specimens and patients studied. Sturm (1986a), who examined sputum samples sent to a clinical laboratory, and Watson *et al.* (1985), who compared patients with cystic fibrosis or other respiratory infection, both found the commonest biotype in the sputum was 2 (37–43%). Oberhofer and Back (1979), on the other hand, found biotype 1 to account for 46%, 2 for 26% and 3 for 20% of isolates from sputum samples they examined.

In our study, biotype 2 was significantly more likely to be resistant to ampicillin, tetracycline and sulphonamide than biotypes 1 and 3, whereas biotype 1 was significantly more likely to be resistant to trimethoprim than biotypes 2 and 3. Three of the five multiresistant isolates were found to contain plasmids, none of which were capable of self-

transfer. We did not investigate the possible presence of larger, transmissible plasmids integrated in the host chromosome. Brunton *et al.* (1986) presented evidence that the small, nonconjugative, β -lactamase-specifying plasmids of *Neisseria gonorrhoeae* and *Haemophilus* spp. are highly related and that phenotypically cryptic plasmids found in several epidemiologically distinct isolates of *H. parainfluenzae* are also related to the β -lactamase plasmids but do not carry transposon A(TnA) sequences. More recently, Martel *et al.* (1987) studied three isolates of biotype 2 from the urogenital tract and found them to contain a β -lactamase-coding 4.6-kb plasmid which was identical to the 4.6-kb "African-type" plasmid found in *N. gonorrhoeae*. These strains of *H. parainfluenzae* could, therefore, serve as a reservoir of antibiotic-resistance plasmids which might then be acquired by *N. gonorrhoeae* or *H. influenzae* present in the urogenital or respiratory tracts.

H. parainfluenzae is generally regarded as a commensal of the respiratory tract and has only occasionally been implicated in local or systemic infections, such as pharyngitis, epiglottitis, otitis media, conjunctivitis, dental abscesses, pneumonia, prostatitis, septicaemia, endocarditis, septic arthritis, meningitis, brain abscess, liver abscess and osteomyelitis (Chow *et al.*, 1974; Chunn *et al.*, 1977; Hand, 1979; Chattopadhyay *et al.*, 1983;

Clairmont *et al.*, 1987; Olk *et al.*, 1987). Rhind *et al.* (1985) suggested that *H. parainfluenzae* is associated with the same clinical spectrum of illness as non-capsulate *H. influenzae* in respiratory infections. A role for *H. parainfluenzae* in non-gonococcal urethritis has been suggested by Fuzi (1980) and Sturm (1986b) but remains to be confirmed by prospective, controlled studies. Like other commensals, *H. parainfluenzae* may be pathogenic in patients who are immunosuppressed, as illustrated by the report (Clairmont *et al.*, 1987) of *H. parainfluenzae* prostatitis in a homosexual man with chronic lymphadenopathy syndrome and HIV infection.

In conclusion, we have shown that *H. parainfluenzae* is carried much less frequently in the vagina than in the male urethra. Biotype 2 is a genital type and is more likely to be resistant to antibiotics than biotypes 1 and 3 which are found mainly in the throat.

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