Epidemiol. Infect. (1993), **111**, 89–98

An analysis of the diversity of *Haemophilus parainfluenzae* in the adult human respiratory tract by genomic DNA fingerprinting

G. R. D. KERR[1], K. J. FORBES[2]*, A. WILLIAMS[2] AND T. H. PENNINGTON[2]

[1] *British Antarctic Survey Medical Unit, Robert Gordons Institute of Technology Survival Centre, King Street, Aberdeen AB2 3BJ*

[2] *Department of Medical Microbiology, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD*

(*Accepted 18 February 1993*)

SUMMARY

A method for typing *Haemophilus* species is described, based on the analysis of genomic DNA from *Haemophilus parainfluenzae*. The DNA was extracted by a rapid method and digested with the restriction enzyme *Bam*HI to provide a characteristic 'fingerprint'. The pattern of fragments in the ranges 1–1·6 kb, 1·6–2 kb and 2–3 kb were used to produce a numerical profile of each isolate. In total 97 isolates were examined; 88 from throat swab material isolated from the 15 members of a British Antarctic Survey base and 9 type strains. Seventy-two of the 88 antarctic isolates were *H. parainfluenzae* and were found to be very diverse, comprising 41 identifiable strains with up to 5 strains being isolated from a single throat swab sample. There was evidence for both carriage and transmission within the isolated community. The technique provided a highly discriminatory method for characterizing *Haemophilus* strains which is suitable for epidemiological studies.

INTRODUCTION

Members of the genus *Haemophilus* are ubiquitous in the human oral cavity and upper respiratory tract and have been isolated from over a quarter of stool specimens [1]. Eight species are currently recognized as commensal in man; *Haemophilus influenzae*, *H. parainfluenzae*, *H. parahaemolyticus*, *H. paraphrohaemolyticus*, *H. aphrophilus*, *H. paraphrophilus*, *H. segnis* and *H. haemolyticus*. Some species show a predeliction for particular anatomical sites, such as *H. aphrophilus* and *H. segnis*, whose normal habitat is the oral cavity [2, 3]. Others are abundant only in certain age groups, *H. influenzae* for instance, being isolated much more frequently from healthy children than adults [4, 5].

While *H. influenzae* is undoubtedly the most important potential pathogen, in health *H. parainfluenzae* accounts for the single largest proportion of *Haemophilus* species in the pharynx at all ages [5] and in the adult oral cavity [6, 7]. Though species other than *H. influenzae* are thought to have little potential for causing infection, the pathogenicity of *H. parainfluenzae* may not be fully recognized because it is often ignored in clinical specimens. Invasive infections caused by *H.*

* Author for correspondence.

parainfluenzae are reported sporadically, the most common being meningitis, endocarditis and pneumonia [8]. Its role in respiratory infection has been reported as indistinguishable from that caused by non-capsulate *H. influenzae* [9, 10], with one observation from the developing world indicating that 10% of haemophilus isolates from the blood of children with lower respiratory infection were *H. parainfluenzae* [11].

Published studies on colonization have centred on *H. influenzae* which has an extremely diverse yet clonal population [11–14], with non-capsulate *H. influenzae* being one of the most diverse organisms known. Carriage studies in healthy children have found up to five different biotypes of *H. influenzae* present at one time compared with only one in healthy adults [5]. In infection, multiple types are rarely observed [15, 16]. Typing schemes for *H. parainfluenzae* are not well developed and studies in healthy subjects have been limited to biotyping, eight types now being recognized [2, 17].

We report here the results of studies on *H. parainfluenzae* carriage in healthy adult subjects living on a British Antarctic Survey base (Signy Station, latitude 61° S) over a period of 8 months, 7 months of which were in isolation from external human contact. Isolates were speciated by biochemical tests and were typed by DNA fingerprints obtained by restriction enzyme digestion of total genomic DNA, resolved by agarose gel electrophoresis.

MATERIALS AND METHODS

Bacterial isolates

Bacteriological samples were collected from each of the overwintering base members (15 men, average age 26 years) at interval of 3–4 weeks during the period March–October 1990. Four sampling times with recovery of *Haemophilus* species from 75% of individuals were studied in detail; March (immediately pre-isolation), April, July and October (all during isolation).

Samples were taken by rubbing a moistened sterile cotton wool swab over the left tonsillar fossa. Note was made of any upper respiratory symptoms, recent antibiotic therapy and smoking habits. The swab was immediately inoculated onto Columbia agar (Oxoid Ltd.) supplemented with Fildes peptic digest (Oxoid, 5% vol/vol) and NAD (Sigma, 10 μg/ml). After overnight growth in a candle extinction jar at 37 °C the mixed growth was swept off with a sterile cotton wool swab, emulsified in 2 ml 7·5% (w/v) glucose-horse serum and freeze dried in glass vials on a Modulyo – 4 K freeze drier (Edwards High Vacuum International, Crawley, UK). These vials were stored and transported in the dark at 4 °C.

Reconstitution of the samples was by rehydration with 1 ml sterile distilled water, plating onto selective chocolate agar containing vancomycin (5 μg/ml), and after overnight growth (37 °C in air + 5% CO_2), haemophilus-like colonies were subcultured onto chocolate agar. Identification was made by their appearance on 7·5% (v/v) defibrinated horse blood agar with V-factor provided by an impregnated disc (Oxoid), noting also the presence of haemolysis. Speciation of strains was based on an X and/or V factor requirement using disks (Oxoid) on nutrient agar, haemolysis, CO_2 requirement for growth at 37 °C and acid production from glucose, sucrose and lactose in test tubes. For those strains giving equivocal results, a tube test for porphyrin production from δ-aminolaevulinic

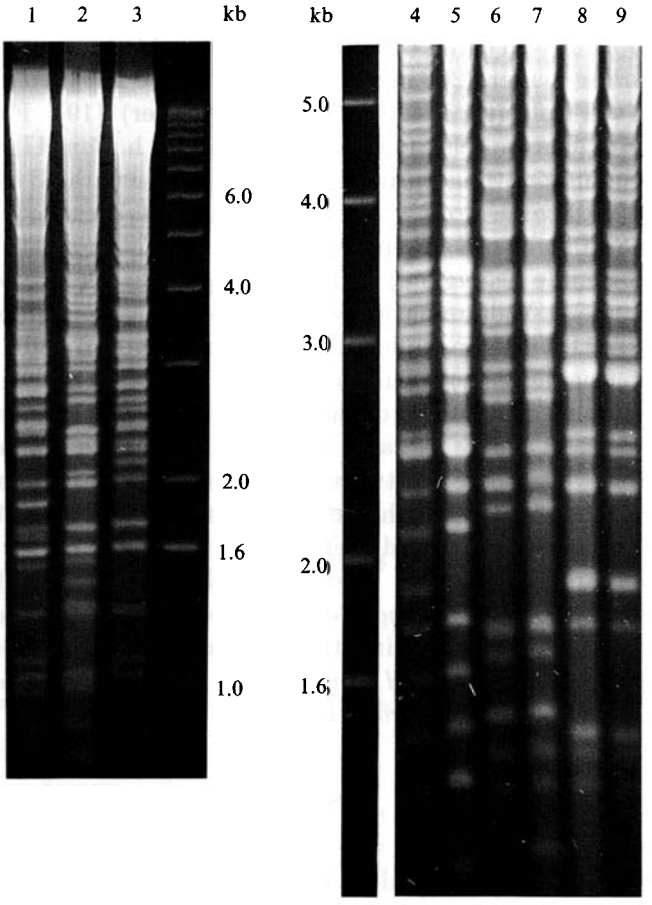

Fig. 1. Electrophoresis gels of DNA from nine strains of *H. parainfluenzae* digested with *Bam*HI. Tracks 1, 2 and 3 are representative fingerprints of unrelated strains produced with a 1 kb-ladder co-loaded. Tracks 4 and 5, 6 and 7, 8 and 9 are three pairs of similar strains, differing by 2, 2 and 1 band respectively.

acid [2] and tests of indole production, urease, β-galactosidase (ONPG) and ornithine decarboxylase activity using an API 10S test strip [18] were performed. Strains were stored in duplicate by both freeze-drying and at -70 °C.

Type strains used in the study for comparison and standardization were *H. parainfluenzae*, Biotype I (NCTC 7857), *H. parainfluenzae*, Biotype II, capsulate, (NCTC 10665), *H. parainfluenzae*, Biotype III (NCTC 11607), *H. parahaemolyticus* (NCTC 8479), *H. paraphrohaemolyticus* (NCTC 10670), *H. aphrophilus* (NCTC 5906), *H. paraphrophilus* (NCTC 10557), *H. segnis* (NCTC 10977) and *H. haemolyticus* (NCTC 10659).

DNA extraction and restriction enzyme digestion

Preparation of total genomic DNA was performed using a microcentrifuge tube method which extracts up to 300 μg bacterial DNA from the growth on a single chocolate agar plate [19]. Restriction enzyme digestion and agarose gel electrophoresis were performed as in Sambrook and co-workers [20] with 5–10 units of enzyme per digest containing between 5 and 30 μg (mean 15 μg) of DNA

in a final volume of 20 μl. For DNA fingerprinting of strains a 1–12 kb DNA size standard with 1 kb spaced rungs (GIBCO-BRL) was added to each digest after the addition of loading buffer (200 mM EDTA (pH 8·0), 0·25% w/v Bromophenol Blue, 25% w/v Ficoll (type 400) in distilled water) [19]. Fragments were separated on 0·8% agarose gels run at 1 v/cm for 18–48 h. *Bam*HI, *Kpn*I and *Xba*I produced discriminatory fingerprints of 10–20 well separated bands in most strains. *Dra*I, *Alu*I, *Eco*RI, *Eco*RV, *Hae*III, *Pst*I and *Taq*I restricted the DNA too frequently and *Sal*I and *Sma*I too infrequently to be of general use. After staining with ethidium bromide (0·5 μg/ml) and destaining in distilled water, the gel was photographed under UV illumination with a Polaroid camera (Fig. 1).

Following *Bam*HI digestion, co-loading with size standard and electrophoresis, the number of fragments between rungs 1–1·6 kb, 1·6–2 kb and 2–3 kb were counted. A graphic representation of the banding pattern was constructed which produced a numerical profile of each isolate and this data was entered into a computer database (Dataease, Software Solutions, Inc.). Pairs of similar isolates were recognized when indistinguishable in at least 2 out of the 3 rungs and was confirmed by running the pair in adjacent lanes without the ladder co-loaded and comparing the banding patterns over their whole length. Isolates with indistinguishable fingerprints were considered to be the same strain. Each strain was given a code number indicating the order of isolation and a letter prefix designating species, namely i, *H. influenzae*; p, *H. parainfluenzae*; ph, *H. parahaemolyticus*; pph, *H. paraphrohaemolyticus*; s, *H. segnis*.

RESULTS

Characterization of isolates

The biochemical tests correctly identified all the type strains and assigned 90% (79/88) of antarctic throat swab isolates to recognized species, 67 being *H. parainfluenzae*. The other species isolated were *H. influenzae* (2), *H. parahaemolyticus* (6), *H. paraphrohaemolyticus* (3) and *H. segnis* (1). Five of the nine isolates displaying anomalous results in one or more character were designated 'atypical' *H. parainfluenzae*. Four of these were weak fermenters of glucose and sucrose but distinguishable from *H. segnis*; one (p27) was CO_2 dependent, but all produced large colonies with strong ornithine decarboxylase activity. The remaining isolate (p35) was strongly CO_2 dependant but distinguishable from *H. paraphrophilus* by its strong ornithine decarboxylase activity and failure to ferment lactose [3].

Restriction enzyme analysis

Complete reproducibility of *Bam*HI fingerprints was found: from repeated DNA preparations from isolates stored at −70 °C for up to 9 months and from isolates serially subcultured six times. No differences were detectable between isolates with indistinguishable *Bam*HI fingerprints when restricted with *Xba*I or *Taq*I. All isolates with indistinguishable fingerprints exhibited the same biochemical characteristics.

To determine the extent of strain diversity an initial survey was performed by fingerprinting up to three haemophilus isolates per sample. Individuals were frequently found to be carrying different species or strains of the same species with different fingerprints at one sampling time. This finding was examined in greater

Table 1. *Diversity of strains carried by subjects A and B at sampling times in March and July by analysis of at least 25 colonies from each sample*

Strains	Sample period: March No. of colonies (%)	Sample period: July No. of colonies (%)
Subject A		
H. parainfluenzae		
p2	9* (35%)	14* (54%)
p36	3 (11%)	
p4	1* (4%)	
p35	1 (4%)	
p38		4 (15%)
p39		3 (11%)
p7		1* (4%)
p40		1 (4%)
H. parahaemolyticus		
ph1	1 (4%)	1* (4%)
ph4	5 (19%)	
H. paraphrohaemolyticus		
pph5	6* (23%)	1 (4%)
pph6		1 (4%)
Total	26 (100%)	26 (100%)
Subject B		
H. parainfluenzae		
p21	11 (39%)	13* (48%)
p23	5 (18%)	6 (22%)
p29	8* (28%)	
p30	3* (11%)	
p22	1 (4%)	
p4		6* (22%)
p41		1 (4%)
H. influenzae		
i1		1 (4%)
Total	28 (100%)	27 (100%)

* Observed in initial small scale examination.

depth by fingerprinting at least 25 colonies each from samples collected in March and July from subjects A and B. This revealed carriage of from 5–8 differentiable haemophilus strains from up to three different species, with the majority of isolates from each sample being *H. parainfluenzae* (Table 1).

Considering the *H. parainfluenzae* strains isolated from all base members over the four sampling periods, 59% (24/41) were isolated only once. Eleven of the 17 strains isolated more than once, were only re-isolated from the same subject and 6 from 2 or more different subjects. The most abundant strain (p2) was isolated a total of 10 times, in 4 people up to 4 times each. Two strains (p2 and p21) were each isolated from the same subject 7 months apart (the duration of the study). Tables 2 and 3 summarise carriage and distribution of strains.

The utility of *Bam*HI fingerprinting is exemplified by the ready detection of

https://doi.org/10.1017/S0950268800056715 Published online by Cambridge University Press

Table 2. *Temporal distribution of* H. parainfluenzae *strains isolated from different subjects*

	Sample period			
	March	April	July	October
Subject	Strains identified			
A†	p2 p4 p35 p36	p2	p2 p7 p38 p39 p40	p2*
B†	p21 p23 p22 p29 p30	p21 p23* p22	p21 p23 p4 p41	p21 p19
C	p2 p34	p2 p11*	p2	a
D	p2	p2 p16*	p2	a
E	p2	a	p10 p3 p18	p10
F	b	p13 p4	p13*	a
G	p26	a	p23*	b
H	p19*	p37*	a	a
I	p28 p27	p28*	p13	p13
J	p26	a	a	a
K	a	a	p9*	p9 p6
L	p33	a	p1 p5	p1 p5
M	p24 p32	p24 p25	p14 p17	p14
N	a	p8	a	a
O	p12 p31	p12 p20	p20 p15	p20

Strain numbers indicate the order of isolation.
* Indicates two colonies sampled with identical fingerprints.
† > 25 colonies examined from each March and July sample of subjects A and B.
a No haemophilus recovered from sample.
b No *H. parainfluenzae* recovered from sample.

https://doi.org/10.1017/S0950268800056715 Published online by Cambridge University Press

Table 3. *Distribution of* H. parainfluenzae *strains amongst the subjects*

Subject	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Number of strains unique to subject	6	5	2	1	3	0	0	1	2	0	2	3	5	1	4
Strains occurring in more than one subject	p2 p4	p4 p19 p23	p2	p2	p2	p4 p13	p23 p26	p19	p13	p26	—	—	—	—	—

three pairs of *H. parainfluenzae* strains that differed by only 1 or 2 bands out of 25 bands in each strain in the range 1–5 kb (Fig. 1). This was confirmed by analysing the restriction pattern produced by two other enzymes; *Kpn*I (similar restriction frequency) and *Taq*I (more frequent restriction). Using *Kpn*I each pair differed by 3 bands or less out of 25, while with *Taq*I only minor differences were observed.

DISCUSSION

Survival rates of antarctic specimens

As strain characterization was undertaken following the reconstitution of the freeze dried specimens, actual survival rates over this period are impossible to determine. The recovery of haemophilus isolates from 75% of all antarctic freeze dried samples is broadly comparable with the prevalence of haemophilus strains found in other similar studies when the systematic losses inherent in the freeze drying process are taken into account [5].

Typing of H. parainfluenzae

The biochemical characterization of the isolates revealed them to be mostly typical *H. parainfluenzae* with a few atypical strains in addition to other species of *Haemophilus*. Biotyping of *H. parainfluenzae* offers limited application. OMP profiles are similar to, but distinguishable from, those of *H. influenzae* and are independent of LPS profile [21]. *H. parainfluenzae* OMP profiles from infant conjunctivitis [22] and OMP/electropherotype (ET) patterns in blood culture isolates from children with lower respiratory infection [11] have also been examined.

DNA fingerprinting of non-typable *H. influenzae* has previously been reported as a quick, reproducible, high resolution technique for identifying a large diversity of bacterial strains [15, 19] that correlates well with findings from protein and rRNA studies [23]. The analysis of fingerprints by the production of a numerical profile for each isolate was essential for the simple handling of the complex patterns produced. The power of the method is illustrated by the three pairs of similar strains which were readily discovered amongst the 44 identifiable *H. parainfluenzae* fingerprints (Fig. 1).

Strain diversity

H. influenzae was isolated from two of 15 individuals but the vast majority (86/88) of haemophilus isolates were only V-dependent. This supports previous work on carriage; though *H. influenzae* is carried in the pharynx of approximately 40% of healthy adults [9], it makes up only 2% of the total haemophilus flora [5].

The maximum number of haemophilus strains identifiable in a single sample in this study (8) is probably a function of the sampling method. Thus, examination of 25 colonies per sample gives a 90% probability of detecting at least 1 member of each of 7 strains, if these are present in equal proportions and sampling is random [24]. The actual proportions of different strains present largely determines the likelihood of detecting each strain, with unequal proportions increasing the sample size required. Not withstanding this qualification, the degree of diversity observed in this study exceeds those noted in other studies; using biotyping of *H. parainfluenzae*, an average of 4 (maximum 5) haemophilus strains per subject were identified from dental plaque when approximately 55 colonies were examined [7]. The reason for this is presumably the superior discrimination of strains which was possible with the DNA fingerprinting method used in this study, as well as any variation in strain diversity with anatomical region.

Carriage and source of diversity

The presence of up to five strains of *H. parainfluenzae* in single samples indicates a degree of diversity, in an anatomically extremely localized area, similar to that of *Escherichia coli* which was found by examining a similar number of colonies from the much more heterogeneous environment of the gut [25]. Examination of the degree of strain localization within the pharynx would be interesting and could include comparison of strains isolated at the same time from the tonsillar fossa on each side.

In the subjects intensively studied here, subject A carried one *H. parainfluenzae* strain and subject B two strains over a prolonged period, with all the other strains only being isolated once.

Two models of carriage can be postulated to explain the apparent ebb and flow in the carriage of different strains in an individual. Isolated strains may be representative of 'resident' and 'transient' strains, the latter never establishing themselves for an extended period. This has been proposed for gut [25] and skin flora [26]. Alternatively, if the actual carried population is very diverse, with some strains being much more abundant than others, then samples which are small in comparison will only identify the most common strains in repeated samplings, rarer strains, though still 'resident' would only occasionally be detected. In this latter model carriage diversity and the persistence time of strains would both be underestimated.

Strains were isolated on more than one occasion not only from individuals over a period of time, as discussed above, but also in different individuals. In this study, 15% (6/41) of *H. parainfluenzae* strains were found in more than one individual. This compares with the 20% of *E. coli* strains shared between subjects in the Antarctic study of Tzabar and Pennington [27], and the 11% of *E. coli* strains shared between family members [28]. The widespread isolation of some strains could be due either to the transfer of strains between subjects during the study period [27], or alternatively, strains with a wide geographical distribution having been independently introduced by more than one subject [28]. Exclusion of the latter possibility could only be approached by more extensive sampling, commencing before any possibility of contact between study members.

The finding that, on repeated sampling 59% of *H. parainfluenzae* strains were isolated only once is comparable with a figure of 86% (147/170) for a study of non-

typable *H. influenzae* from clinical specimens derived from a population of 500000 [29]. This continued isolation of new strains throughout the duration of this study is noteworthy. That the subjects here were in a small closed community excludes an external origin for these new strains. Although members of the genus *Haemophilus* are naturally transformable, the extensive genotypic heterogeneity observed between strains in this study probably could not have arisen as a result of the horizontal transfer of genetic material between strains. Any such recombinants would not be grossly different from their parental strains. Three different pairs of similar strains were, however, noted and these deserve further study. It seems more probable that the continuing occurrence of new strains was due to a diversity of strains carried by most individuals, which had previously not been detected. The discriminatory power of the methods used here largely contributed to this finding. In the light of this, future studies will either have to characterize many more isolates from each sample period to detect rarer strains, or alternatively use a sampling method which is able to, at least qualitatively, determine the presence of particular strains in subjects. Given the great genetic diversity of the strains examined here the latter approach may be feasible if suitable molecular probes can be designed; PCR amplification of target genes and analysis of the product by restriction enzyme polymorphisms would be one possibility.

This is the first report of the use of a highly discriminatory method for the typing of *H. parainfluenzae*. It has established that, like the well studied *H. influenzae*, this more abundant and widespread species shows extensive genetic diversity. Further work done with these isolates in this laboratory (data not shown) supports the consensus of recent reports [30, 31] that *H. parainfluenzae* is a very heterogeneous grouping and perhaps not a natural taxon.

ACKNOWLEDGEMENTS

This work was supported by the British Antarctic Survey. We wish to thank Alison Williams for her expert technical assistance throughout and the Department of Medical Illustration, Foresterhill, for their help in preparing the figures.

REFERENCES

1. Palmer GG. *Haemophilus* in faeces. J Med Microbiol 1981; **14**: 147–50.
2. Kilian M. A taxonomic study of the genus *Haemophilus* with the proposal of a new species. J Gen Microbiol 1976; **93**: 9–62.
3. Kilian M, Biberstein EL. Genus II. *Haemophilus*. In: Krieg NR, Holt JH, eds. Bergey's manual of systematic bacteriology. Vol I. Baltimore: Williams and Wilkins, 1984: 558–69.
4. Kilian M, Heine-Jensen J, Bülow P. *Haemophilus* in the upper respiratory tract of children. Acta Path Microbiol Scand 1972; **80**: 571–8.
5. Kuklinska D, Kilian M. Relative proportions of *Haemophilus* species in the throat of healthy children and adults. Eur J Clin Microbiol 1984; **3**: 249–52.
6. Sims W. Oral haemophili. J Med Microbiol 1970; **3**: 615–25.
7. Kilian M, Schiøtt CR. Haemophili and related bacteria in the human oral cavity. Archs Oral Biol 1975; **20**: 791–6.
8. Albritton WL. Infections due to *Haemophilus* species other than *H. influenzae*. Ann Rev Microbiol 1982; **36**: 199–216.
9. Turk DC, May JR. *H. influenzae*. Its clinical importance. London: English University Press, 1967: 11.

10. Rhind GB, Gould GA, Ahmad F, Croughan MJ, Calder MA. *Haemophilus parainfluenzae* and *H. influenzae* respiratory infection: comparison of clinical features. BMJ 1985; **291**: 707–8
11. Weinberg GA, Ghafoor A, Ishaq Z et al. Clonal analysis of *Haemophilus influenzae* isolated from children from Pakistan with lower respiratory tract infections. J Infect Dis 1989; **160**: 634–43.
12. Porras O, Caugant DA, Gray B, Lagergård T, Levin BR, Svanborg-Edén C. Difference in structure between type b and nontypable *Haemophilus influenzae* populations. Infect Immun 1986; **53**: 79–89.
13. Musser JM, Kroll, JS, Granoff DM et al. Global genetic structure and molecular epidemiology of encapsulated *Haemophilus influenzae*. Rev Infect Dis 1990; **12**: 75–111.
14. Pennington TH. *Haemophilus* species and clones. Rev Med Microbiol 1993; **4**: In press.
15. Loos BG, Bernstein JM, Dryja DM, Murphy TF, Dickinson DP. Determination of the epidemiology and transmission of nontypeable *Haemophilus influenzae* in children with otitis media by comparison of total genomic DNA restriction fingerprints. Infect Immun 1989; **57**: 2751–7.
16. Groeneveld, K, Van Alphen L, Eijk PP, Visschers G, Jansen HM, Zanen HC. Endogenous and exogenous reinfections by *Haemophilus influenzae* in patients with chronic obstructive pulmonary disease: the effect of antibiotic treatment on persistence. J Infect Dis 1990; **161**: 512–7.
17. Doern GV, Chapin KC. Determination of biotypes of *Haemophilus influenzae* and *Haemophilus parainfluenzae*; a comparison of methods and description of a new biotype (VIII) of *H. parainfluenzae*. Diagn Microbiol Infect Dis 1987; **7**: 269–72.
18. Watson KC, Kerr EJC, Baillie M. Comparison of API-10S and tube tests for biotyping *H. influenzae*. J Clin Pathol 1986; **39**: 692–693
19. Forbes KJ, Bruce KD, Jordens JZ, Ball A, Pennington TH. Rapid methods in bacterial DNA fingerprinting. J Gen Microbiol 1991; **137**: 2051–8.
20. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning; a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
21. Roberts MC, Mintz CS, Morse SA. Characterisation of *Haemophilus parainfluenzae* strains with low-M_r or ladder-like lipopolysaccharides. J Gen Microbiol 1986; **132**: 611–6.
22. Roberts MC, Bell TA, Sandstrom KI, Smith AL, Holmes KK. Characterisation of *Haemophilus* spp isolated from infant conjunctivities. J Med Microbiol 1986; **21**: 219–24.
23. Bruce KD, Jordens JZ. Characterisation of noncapsulate *Haemophilus influenzae* by whole cell polypeptide profiles, restriction endonuclease analysis, and rRNA gene restriction patterns. J. Clin Microbiol 1991; **29**: 291–6.
24. Hedges AJ, Howe K, Linton AH. Statistical considerations in the sampling of *Escherichia coli* from intestinal sources for serotyping. J Appl Bacteriol 1977; **43**: 271–80.
25. Caugant DA, Levin BR, Selander RK. Genetic diversity and temporal variation in the *E. coli* population of a human host. Genetics 1981; **98**: 467–90.
26. Maggs AF, Pennington TH. Temporal study of *Staphylococcal* species on the skin of human subjects in isolation and clonal analysis of *Staphylococcus capitis* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. J Clin Microbiol 1989; **27**: 2627–32.
27. Tzabar Y, Pennington TH. The population structure and transmission of *E. coli* in an isolated human community; studies on an Antarctic base. Epidemiol Infect 1991; **107**: 537–42.
28. Caugant DA, Levin BR, Selander RK. Distribution of multilocus genotypes of *Escherichia coli* within and between host families. J Hyg 1984; **92**: 377–84.
29. Bruce KD, Pennington TH. Clonal analysis of non-typable *Haemophilus influenzae* by sodium dodecyl sulphate-polyacrylamide gel electrophoresis of whole cell polypeptides. J Med Microbiol 1991; **34**: 277–83.
30. Pohl S. DNA relatedness among members of *Haemophilus*, *Pasteurella* and *Actinobacillus*. In: Kilian M, Frederiksen W, Biberstein EL, eds. *Haemophilus*, *Pasteurella* and *Actinobacillus*. London: Academic Press. 1981: 245–53.
31. Dewhirst FE, Paster BJ, Olsen I, Frazer GJ. Phylogeny of 54 representative strains of species in the family *Pasteurellaceae* as determined by comparison of 16S rRNA sequences. J Bacteriol 1992; **17**: 2002–13.