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Esterase electrophoresis: a molecular tool for studying the epidemiology of *Branhamella catarrhalis* nosocomial infection

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SUMMARY

A new epidemiologic typing method based on electrophoresis of esterases had been developed for differentiating between clinical isolates of *Branhamella catarrhalis*. Twenty-two epidemiologically significant strains obtained from three Chest Units, a Paediatric Intensive Care Unit and a Paediatric Unit were compared with 54 randomly selected strains and 4 reference strains, including the species type strain, ATCC 25238. Thirty-four distinct zymotypes were characterized by polyacrylamide-agarose gel electrophoresis of the 80 strains. One infrequent zymotype was found in 2 neonates and another in 2 adults with nosocomial bronchopulmonary infections, suggesting the nosocomial spread of 2 outbreak strains of *B. catarrhalis*. A more frequent zymotype was isolated from 3 neonates with nosocomial bronchopulmonary infection and from 2 children with nosocomial rhinopharyngitis. The remaining 12 epidemiologically significant strains were of varied zymotypes. This work demonstrates that esterase electrophoresis is a suitable, readily reproducible, stable typing system applicable to the wide range of strains found in *B. catarrhalis* nosocomial infections.

INTRODUCTION

Branhamella catarrhalis, a commensal of the oropharynx, is increasingly recognized as a human pathogen. This gram-negative diplococcus commonly causes respiratory infections in adults with chronic lung disease and otitis media in children (1–4). Nosocomial spread of an outbreak strain of *B. catarrhalis* has been suggested, but was not confirmed because of the lack of a typing system to establish strain relatedness (5–7). A recent nosocomial outbreak of respiratory illness due to *B. catarrhalis*, was confirmed by restriction endonuclease analysis (8). However, electrophoretic typing of esterases has been shown to provide rapid, easy discrimination for epidemiological screening (9–12). We have, therefore, used this method to differentiate clinical isolates of *B. catarrhalis*.

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MATERIALS AND METHODS

Strains and clinical features. Seventy-six clinical isolates and four reference strains of *B. catarrhalis* were studied. The reference strains were: the type strain of the species *B. catarrhalis* ATCC 25238 and three strains from the Laboratoire des *Neisseria*, Institut Pasteur, Paris, France: LNP 4122, LNP 4130 and LNP 4663. Twenty-two of the clinical isolates appeared to be epidemiologically significant (Table 1) and 54 were not. Ten of the epidemiologically significant strains were from adults in three Chest Units on the same floor of one hospital, 10 were from neonates in the Paediatric Intensive Care Unit and 2 were from children in a Paediatric Unit of another hospital. Three of the 10 Chest Unit strains isolated between February and April 1987, were found in the sputum or endotracheal aspirates of 3 patients with nosocomial bronchopulmonary infection, and 7 were found in the nasopharyngeal swabs from 7 members of staff (6 with nasopharyngitis of unknown etiology and 1 asymptomatic) who had cared for the patients. The 10 Paediatric Intensive Care Unit strains were found in endotracheal aspirates from 8 neonates between November 1987 and January 1988. Three of these strains were from the same neonate, taken 4 and 14 days apart. The child was suffering from bronchopulmonary colonization following bronchopulmonary infection. Six of the remaining neonates had nosocomial bronchopulmonary infections and one had colonization. Each neonate was in an individual small room and the same medical staff cared for all the neonates. The two Paediatric Unit strains were taken from the inferior nasal meatus of two children with nosocomial purulent nasopharyngitis of unknown origin; they shared the same room on September 22 1988. All the epidemiological strains produced β-lactamase. The 54 epidemiologically non-significant strains were randomly selected from the bronchopulmonary or nasopharyngeal secretions of 54 patients with bronchopulmonary infection or colonization over a 3-year period (November 1985 to November 1988). These patients were from 17 different units in 2 hospitals in the same town. Forty-six of these 54 strains produced β-lactamase. Bronchopulmonary infection was defined using strict criteria: presence of polymorphonuclear leucocytes and Gram-negative intra- and extra-cellular diplococci on the sputum Gram stain, pure or predominant culture $> 10^7$ CFU per ml of expectorate, compatible clinical syndrome. A nosocomial infection could only be diagnosed by the onset of symptoms > 72 h after admission.

Identification of B. catarrhalis. Isolates were identified as *B. catarrhalis* on the basis of Gram stain, characteristic growth and biochemical reactions (13). β-lactamase was detected using nitrocefin disks (Cefinase, BioMerieux).

Preparation of bacterial extracts. Bacteria were grown at 37 °C in L broth (14) with constant agitation for 18 h and collected by centrifugation. The pellets were washed with 60 mM Tris glycine buffer pH 8·7, resuspended in the same buffer and disrupted by sonication for 18 min at 4 °C. The crude extract supernatants, containing 40–60 mg protein per ml, were stored at -20 °C until used (15).

Electrophoresis. Horizontal slab gel electrophoresis was performed according to the method of Uriel (16) in a composite polyacrylamide agarose gel (7 and 1·4%, respectively) in a discontinuous Tris glycine buffer pH 8·7 at a constant voltage (7 V/cm) until the bromophenol blue marker had run 13 cm. The relative mobility

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Table 1. *Principal features of the 22 clinically epidemiological strains*

No. of strain	Date of isolation	Sample	Origin	Ward	Clinical syndrome	No. of zymotype
	1987					
1	16 Feb.	Sputum	Adult 1	Chest Unit 1	BPI	2
2	19 Feb.	Nasopharyngeal swab	Staff member 1	Chest Unit 1	Nasopharyngitis	18
3	2 Mar.	Endotracheal aspirate	Adult 2	CICU	BPI	33
4	5 Mar.	Nasopharyngeal swab	Staff member 2	CICU	Nasopharyngitis	21
5	5 Mar.	Nasopharyngeal swab	Staff member 3	CICU	Nasopharyngitis	20
6	12 Mar.	Nasopharyngeal swab	Staff member 4	Chest Unit 2	Nasopharyngitis	19
7	12 Mar.	Nasopharyngeal swab	Staff member 5	Chest Unit 2	Nasopharyngitis	32
8	12 Mar.	Nasopharyngeal swab	Staff member 6	Chest Unit 2	Nasopharyngitis	31
9	12 Mar.	Nasopharyngeal swab	Staff member 7	Chest Unit 2	Asymptomatic	17
10	14 Mar.	Endotracheal aspirate	Adult 3	Chest Unit 2	BPI	33
11	12 Nov.	Endotracheal aspirate	Neonate 1	PICU	BPC	7
12	16 Nov.	Endotracheal aspirate	Neonate 1	PICU	BPC	6
13	23 Nov.	Endotracheal aspirate	Neonate 1	PICU	BPI	6
14	24 Nov.	Endotracheal aspirate	Neonate 2	PICU	BPC	26
15	1 Dec	Endotracheal aspirate	Neonate 3	PICU	BPI	6
16	16 Dec.	Endotracheal aspirate	Neonate 4	PICU	BPI	31
17	17 Dec.	Endotracheal aspirate	Neonate 5	PICU	BPI	22
18	26 Dec.	Endotracheal aspirate	Neonate 6	PICU	BPI	22
	1988					
19	2 Jan.	Endotracheal aspirate	Neonate 7	PICU	BPI	22
20	9 Jan.	Endotracheal aspirate	Neonate 8	PICU	BPI	25
21	22 Sept.	Inferior nasal meatus swab	Children 1	Pediatric unit	Nasopharyngitis	22
22	22 Sept.	Inferior nasal meatus swab	Children 2	Pediatric unit	Nasopharyngitis	22

BPI, bronchopulmonary infection; BPC, bronchopulmonary colonization; CICU, Chest Intensive Care Unit; PICU, Pediatric Intensive Care Unit.

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(M_F value) is the distance moved by the esterase band as a percentage of the distance moved by the dye front. M_F values were compared by running the bacterial extracts side by side in the same gel; the order of the extracts was changed in some experiments (17).

Characterization of esterases. The estarases were stained on the gel (18, 19) using the following specific substrates: α-naphthyl acetate, β-naphthyl acetate, indoxyl acetate, α-napthyl butyrate and β-naphthyl butyrate (Sigma). The sensitivity of esterases to diisopropylfluorophosphate (DFP) (10^{-3} M) was also tested.

RESULTS

Characterization of esterase bands

Four main kinds of esterase bands were defined by their activity towards synthetic substrates and their sensitivity or resistance to DFP (Table 2); they were numbered B_1–B_4 in order of decreasing mobility towards the anode. Two additional bands hydrolysing α- or β-naphthyl acetate were detected in some strains.

Electrophoretic esterase typing

The 80 *B. catarrhalis* strains could be divided into 34 zymotypes (Fig. 1). Some zymotypes were isolated more frequently than other: zymotype 21 (15 isolates), zymotype 22 (10 isolates), zymotype 20 (5 isolates), zymotype 2, 6 and 18 (4 isolates). Twenty-one zymotypes were found in single strains. Zymotypes 17–23 were closely related and distinguished by the mobility of esterase B_4, which must be precisely measured using indoxyl acetate as substrate.

The four reference strains belonged to four distinct zymotypes: zymotype 15 for strain ATCC 25 238, zymotype 22 for strain LNP 4122, zymotype 21 for strain LNP 4130 and zymotype 1 for strain LNP 4663.

Of the strains that seemed epidemiologically significant (Table 1), zymotype 6 was isolated from 2 neonates with nosocomial bronchopulmonary infections which occurred 8 days apart, zymotype 22 was isolated from 3 neonates with bronchopulmonary infections which occurred 9 and 16 days apart. Zymotype 22

Table 2. *Characteristics of the esterase bands of* B. catharrhalis

	Substrates* hydrolysed†					
	αNA	αNB	IA	βNA	βNB	DFP‡ (10^{-3} M)
Esterase B_1	±	++	−	−	−	R
Esterase B_2	+	+	±	−	−	S
Esterase B_3	++	++	++	++	++	S
Esterase B_4	+	−	+	+	−	S
Additional αa esterase	+	−	−	−	−	S
Additional βa esterase	−	−	−	+	−	S

* αNA, α-naphthyl acetate; αNB, α-naphthyl butyrate; IA, indoxyl acetate; βNA, β-naphthyl acetate; βNB, β-naphthyl butyrate.

† relative intensity of staining: ++ > + > ±. Undetected, −.

‡ R, resistant; S, sensitive.

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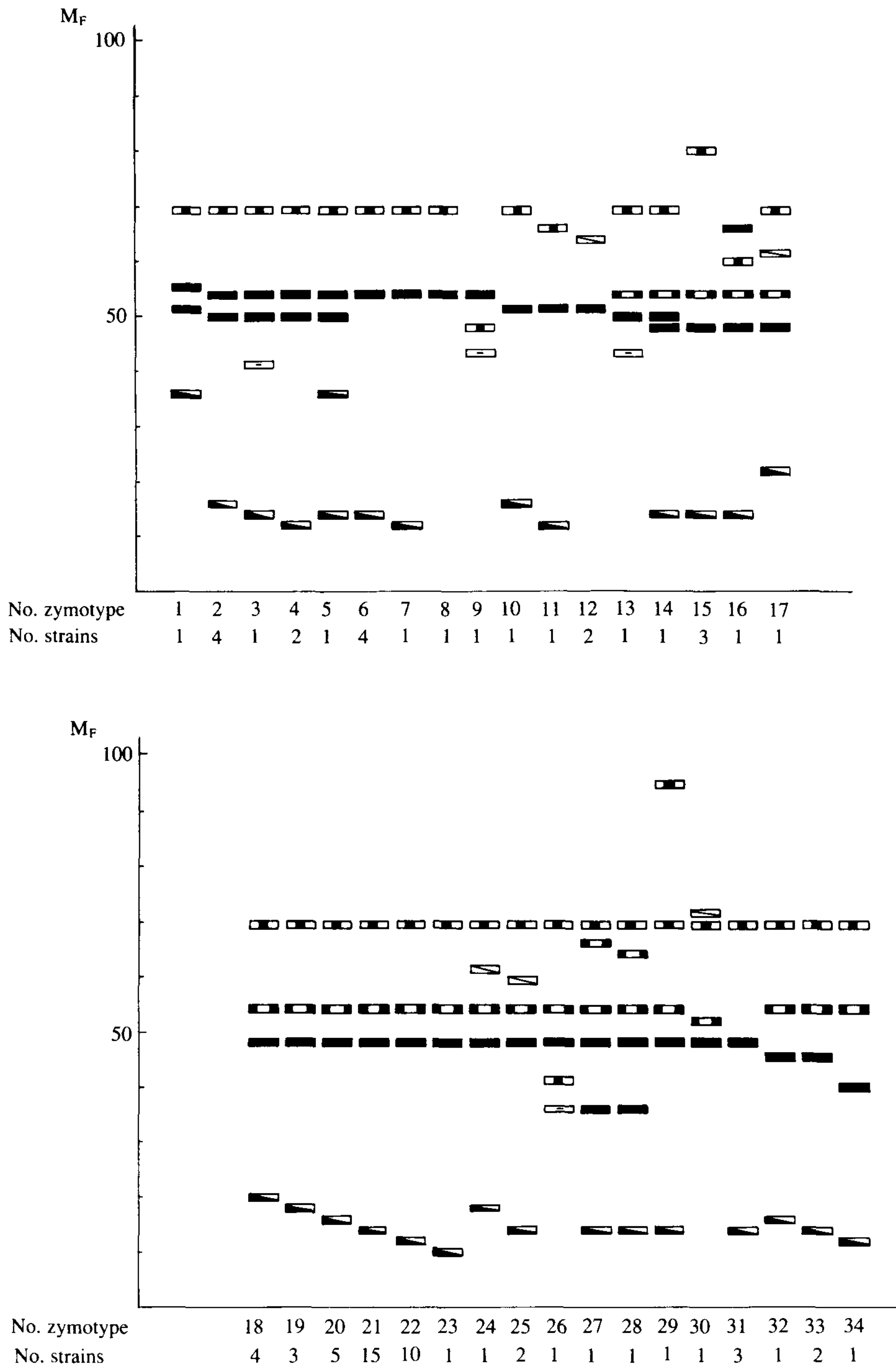

Fig. 1. Diagrammatic representation of esterase patterns of the 80 strains of *B. catarrhalis* grouped 1 according to zymotypes. ▭, esterase B_1; ▭, esterase B_2; ▬, esterase B_3; ▭, esterase B_4; ▭, additional esterase hydrolysing α-naphthyl acetate; ▭, additional esterase hydrolysing β-naphthyl acetate.

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was also isolated from the 2 childrens with nosocomial purulent nasopharyngitis. Zymotype 33 was isolated from 2 adults (patients 2 and 3) with bronchopulmonary infections which occurred 12 days apart. Both patients had been in the same Chest Intensive Care Unit. The adult 2 was transferred from the Chest Intensive Care Unit to the Chest Unit 2 on 16 February 1987. All the other epidemiologically-significant strains were of different zymotypes.

DISCUSSION

Outbreaks of infection due to *B. catarrhalis* on hospital wards have been well documented (5–8). A typing system suitable for epidemiological surveys of these cases must be readily reproducible, stable, and applicable to the wide range of strains to be typed on the basis of a genetic property (20). It must also be inexpensive. Restriction endonuclease electrophoretic analysis of DNA has been successfully used for the epidemiological fingerprinting of *B. catarrhalis* (8, 21). However, this method has a limited application because of the large number of bands and the poor resolution obtained in the gels. Neither electrophoresis of outer membrane proteins (22) nor electrophoresis of total proteins (J. F. Lefebvre, personal communication) provide sufficient discrimination because of the small number of distinct patterns detected. Electrophoretic typing of esterases, which has the advantage of detecting a variety of enzyme bands using five substrates, appears to provide sufficient discrimination for the epidemiological screening of *Escherichia coli* (9), motile *Aeromonas* (10, 11), *Enterobacter sakazakii* (23) and *Serratia marcescens* (12, 24). The 80 *B. catarrhalis* strains studied could be divided into 34 zymotypes. However some zymotypes (nos. 2, 6, 18, 20, 21 and 22) were found in several strains. The reproducibility of the method was established by numerous replicate assays in which the esterase bands of the infecting isolates were compared to those of reference strains placed in adjacent slots on the same gel.

The fact that 2 strains from 2 neonates (neonates 1 and 3) with nosocomial bronchopulmonary infections are of zymotype 6, an infrequent zymotype, confirms the nosocomial spread of an outbreak strain of *B. catarrhalis* (Table 1). The same conclusion may be reached for adults 2 and 3, where both the 2 strains isolated were of zymotype 33. The isolation of 3 strains of zymotype 22 from neonates 5, 6 and 7 with nosocomial bronchopulmonary infection, and of 2 strains of zymotype 22 from the 2 children with nosocomial nasopharyngitis appears to be of little value for establishing *B. catarrhalis* as a transmitted nosocomial pathogen because zymotype 22 is a frequent zymotype in our region. Many of the strains that seemed clinically epidemiologically significant are in fact of different zymotypes. The first and second strains responsible for colonization in neonate 1 are of zymotypes 7 and 6, respectively; the third strain, which was responsible for infection, is of zymotype 6. There is no correlation between the zymotype and the presence or the absence of β-lactamase.

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REFERENCES

1. Denamur E, Suermondt G, Laurans G, Muir JF, Orfila J. Role croissant de *Branhamella catarrhalis* en pathologie bronchopulmonaire de l'adulte. Presse Med 1987; **16**: 1202.
2. Denamur E, Suermondt G, Laurans G, Orfila J, Riou JY. Les infections à *Branhamella catarrhalis*. Semaine Hôp Paris 1988; **64**: 63–70.
3. Hager H, Verghese A, Alvarez S, Berk SL. *Branhamella catarrhalis* respiratory infections. Rev Infect Dis 1987; **9**: 1140–9.
4. Van Hare GF, Shurin PA, Marchant CD, Cartelli NA, Johnson CE et al. Acute otitis media caused by *Branhamella catarrhalis*: biology and therapy. Rev. Infect Dis 1987; **9**: 16–27.
5. Haddad J, Le Faou A, Simeoni U, Messer J. Hospital-acquired bronchopulmonary infection in premature infants due to *Branhamella catarrhalis*. J Hosp Infect 1986; **7**: 301–2.
6. Ahmad F, McLeod DT, Power JT, Calder MA. *Branhamella catarrhalis* prevalence in a hospital population. J Hosp Infect 1985; **6**: 71–4.
7. Denamur E, Suermondt G, Debroca A, Defouilloy C, Laurans G, et al. Infections bronchopulmonaires nosocomiales à *Branhamella catarrhalis* en milieu de réanimation. Agressol. In press.
8. Patterson TF, Patterson JE, Masecar BL, Barden GE, HierHolzer WJ, Zervos MJ. A nosocomial outbreak of *Branhamella catarrhalis* confirmed by restriction endonuclease analysis. J Infect Dis 1988; **157**: 996–1001.
9. Goullet Ph, Picard B. Typage électrophorétique des esterases d'*Escherichia coli* au cours de septicémies. Presse Med 1984; **13**: 1079–81.
10. Picard B, Goullet Ph. Esterase electrophoresis: a new epidemiological screening test for *Aeromonas hydrophila* hospital infection. J Hosp Infect 1984; **5**: 335–7.
11. Picard B, Goullet Ph. Epidemiological complexity of hospital *Aeromonas* infections revealed by electrophoretic typing of esterases. Epidemiol Infect 1987; **98**: 5–14.
12. Picard B, Bruneau B, Goullet Ph. Demonstration of an outbreak of *Serratia marcescens* infections in a medical intensive care unit by esterase electrophoretic typing. J Hosp Infect 1988; **11**: 194–5.
13. Riou JY, Guibourdenche M. *Branhamella catarrhalis*: New methods of bacterial diagnosis. Drugs 1986; **31**: 1–6.
14. Lennox ES. Transduction of linked genetic characters of the host by bacteriophage P1. Virol 1955; **1**: 190–206.
15. Goullet Ph. An esterase zymogram of *Escherichia coli* J Gen Microbiol 1973; **77**: 27–35.
16. Uriel J. Methode d'électrophorèse dans des gels d'acrylamide-agarose. Bul Soc Chim Biol 1966; **48**: 969–82.
17. Goullet Ph, Picard B. A two-dimensional electrophoretic profile for bacterial esterases. Electrophoresis 1985; **6**: 132–5.
18. Lawrence SH, Melnick PJ, Weimer HE. A comparison of serum proteins and enzymes by starch-gel electrophoresis. Proc Soc Experim Biol Med 1960; **105**: 572–5.
19. Uriel J. Caractérisation des cholinestérases et d'autres estérases carboxyliques après électrophorèse et immunoélectrophorèse en gélose (application à l'étude des estérases du sérum humain normal). Ann Inst Pasteur 1961; **101**: 104–19.
20. Aber RC, Mackel DC. Epidemiologic typing of nosocomial microorganisms. Amer J Med 1981; **70**: 899–905.
21. Dickinson DP, Loos BG, Dryja DM, Bernstein JM. Restriction fragment mapping of *Branhamella catarrhalis*: a new tool for studying the epidemiology of this middle ear pathogen. J Infect Dis 1988; **158**: 205–8.

https://doi.org/10.1017/S0950268800030946 Published online by Cambridge University Press

22. Bartos LC, Murphy TF. Comparison of the outer membrane proteins of 50 strains of *Branhamella catarrhalis*. J Infect Dis 1988; **158**: 761–5.
23. Goullet Ph, Picard B. Characterization of *Enterobacter cloacae* and *E. sakazakii* by electrophoretic polymorphism of acid phosphatase, esterases and glutamate, lactate and malate dehydrogenases. J Gen Microbiol 1986; **132**: 3105–12.
24. Goullet Ph. Characterization of *Serratia marcescens*. *S. liquefaciens*, *S. plymuthica* and *S. marinorubra* by the electrophoretic patterns of their esterases. J Gen Microbiol 1978; **108**: 275–81.

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