High ampicillin resistance in different biotypes and serotypes of *Haemophilus influenzae* colonizing the nasopharynx of healthy school-going Indian children

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Haemophilus influenzae is one of the main causes of otitis media, sinusitis, meningitis, pneumonia and septicaemia in children, and the development of ampicillin resistance in *H. influenzae* is a cause of serious concern. The aim of the present study was to determine the prevalence of ampicillin resistance in *H. influenzae* colonizing the nasopharynx of school-going healthy North Indian children, and to compare the distribution of different biotypes and serotype b in this population. A total of 2400 school-going healthy children from 45 rural and 45 urban schools were enrolled. Nasopharyngeal swabs were collected from the children and cultured. *H. influenzae* was isolated from 1001 (41.7 %) of the 2400 nasopharyngeal swabs collected. All these *H. influenzae* isolates were biotyped and serotyped, and their antibiotic susceptibility tested. All eight biotypes were present in this population. The most prevalent biotypes were I (19.6 %), II (16.8 %) and III (25.0 %). Of the 1001 isolates, 316 (31.6 %) were *H. influenzae* type b and 685 (68.4 %) were non-type b *H. influenzae*, and 22.9 % were resistant to ampicillin, 41.9 % to chloramphenicol, 27.5 % to erythromycin and 67.3 % to co-trimoxazole. Of the 316 *H. influenzae* type b isolates, 44.0 % were ampicillin resistant, while only 13.1 % non-type b *H. influenzae* isolates were ampicillin resistant. Of the 229 ampicillin-resistant *H. influenzae* isolates, 196 (85.6 %) were positive for β -lactamase; 93.4 % (214/229) were biotypes I, II and III, of which 49 % were biotype I, 27.9 % were type II and 16.6 % were type III. Most of the strains belonging to biotypes III–VIII were ampicillin sensitive. Ampicillin resistance is significantly more common in biotype I and serotype b than in other biotypes and serotypes.

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INTRODUCTION

Haemophilus influenzae asymptotically colonizes the nasopharynx of healthy individuals, and causes systemic disease and mucous membrane infections. Eight biotypes and six serotypes are used as epidemiological markers for studying the pattern of colonization of *H. influenzae* and to identify the strains of bacterium commonly known to be pathogenic (Alrawi *et al.*, 2002). For instance biotype I and serotype b are commonly associated with meningitis in children, and biotypes II and III are commonly associated with upper respiratory tract infections (Pittman, 1931; Kilian, 1976; Gratten, 1983).

Ampicillin/amoxycillin was the empirical treatment for *Haemophilus* disease until the recent past. Development of ampicillin resistance in causative organisms led to use of third-generation cephalosporins (e.g. ceftriaxone) as empirical drugs. Resistance to ampicillin results from the production of a β -lactamase and alteration of the antibiotic target, penicillin-binding protein (Markowitz, 1980;

Mendelman *et al.*, 1984). Treatment problems linked to the prevalence of β -lactam resistance are compounded by the frequency of cross resistance to many other antibiotics (Talon *et al.*, 2000). The high cost of cephalosporins and the development of drug resistance due to irrational use of antimicrobials are two important limiting factors in the use of cephalosporins in the peripheral level health care system, in developing countries. Currently, in Indian hospitals co-trimoxazole is recommended for the treatment of children with respiratory tract infection at primary health centres. Children presenting with meningitis are treated with third-generation cephalosporins (e.g. ceftriaxone). Continuous monitoring of the antimicrobial resistance patterns of *H. influenzae* is recommended for cost-effective treatment of these infections. Vaccination against *H. influenzae* is usually not carried out for children in our setting. Isolation of *H. influenzae* from cerebrospinal fluid and blood in peripheral hospitals of developing countries may not be possible due to the lack of adequate lab facilities at these hospitals. Hence, the monitoring of nasopharyngeal *H. influenzae* as a

surrogate marker for invasive and locally invasive *H. influenzae* seems an attractive option (Das *et al.*, 2002). In the present study, we aimed to characterize the *H. influenzae* serotypes and biotypes, and looked for their association with ampicillin resistance. An effort was also made to decipher the role of β -lactamase in the development of ampicillin resistance.

METHODS

Study population. From July 2000 to November 2002, a total of 2400 nasopharyngeal specimens were collected from normal school-going children aged between 5 and 10 years, after obtaining an informed written consent from the parents of the child. The mean age of enrolled children was 6.72 years. A total of 90 randomly selected (45 rural and 45 urban) government primary schools, from the Lucknow district of Uttar Pradesh, a Northern state of India, were enrolled. A uniform distribution of rural and urban, and male and female population was ensured.

Sample collection and transport. A calcium alginate tipped swab on a flexible aluminium wire (Hardwood Product Company) was used for the collection of nasopharyngeal specimens. Swabs were transported in STGG (skimmed milk, tryptone, glycerol and glucose) transport medium to the microbiology lab within a few hours.

Bacterial isolation and antibiotic susceptibility test (AST). Nasopharyngeal swabs were plated on chocolate agar with bacitracin ($300 \mu\text{g ml}^{-1}$). The plates were incubated at 37°C in a candle extinction jar, and growth was examined after 24 h. *H. influenzae* isolates were identified by their typical colony morphology, and X and V factor requirement, as described by Jain *et al.* (2005) and Doern (1995). AST was carried out on Haemophilus test medium by a disc diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines (NCCLS, 2000). Four drugs were tested: chloramphenicol (30 μg disc), erythromycin (15 μg disc), co-trimoxazole (2.5 μg trimethoprim and 23.5 μg sulfamethoxazole discs) and ampicillin (30 μg disc).

Determination of β -lactamase. All the ampicillin-resistant strains and 100 randomly selected ampicillin-sensitive strains were tested for β -lactamase production by a nitrocefin disc test (cefinaise; Becton Dickinson Microbiology).

Serotyping. The strains were serotyped by a slide agglutination test, using monospecific antisera for type b (Denka Seiken). Serotyping of all the isolates was done and strains were divided in two groups: *H. influenzae* type b and non-type b *H. influenzae*.

Biotype analysis. Biotypes were assigned to all the *H. influenzae* isolates by assessing their ability to produce urease, indole and ornithine decarboxylase using locally prepared media (Kilian, 1976; Gratten, 1983; Sottnek & Albritton, 1984). Briefly, the indole test was performed by growing each isolate in trypticase soy broth supplemented with NAD ($5 \mu\text{g ml}^{-1}$) and haemin ($5 \mu\text{g ml}^{-1}$). After 18 h growth at 37°C , Kovac reagent was added and the reaction was interpreted as described by Kilian (1976). The urease test was performed on Christensen's urea agar supplemented with NAD ($5 \mu\text{g ml}^{-1}$) and haemin ($5 \mu\text{g ml}^{-1}$). Ornithine decarboxylase activity was assessed on Moeller decarboxylase base supplemented with NAD ($5 \mu\text{g ml}^{-1}$) and haemin ($5 \mu\text{g ml}^{-1}$).

Statistical analysis. Statistical analysis was performed using the STATA 8.2 package, version 6.0.

RESULTS

A total of 1001 (41.7%) isolates of *H. influenzae* were present in 2400 nasopharyngeal specimens from healthy school-going children. Of these isolates, 22.9% were resistant to ampicillin, 41.9% to chloramphenicol, 27.5% to erythromycin and 67.3% to co-trimoxazole. Of the 229 ampicillin-resistant *H. influenzae* isolates 196 (85.6%) were positive for β -lactamase. None of the ampicillin-sensitive strains were positive for β -lactamase. Co-resistance with other antimicrobials in β -lactamase positive and β -lactamase negative, ampicillin-resistant and ampicillin-sensitive strains is shown in Table 1. Co-resistance with other antimicrobials was significantly higher in β -lactamase-producing strains (Table 1).

The distribution of all biotypes, stratified as type b and ampicillin resistant, is shown in Table 2. All eight biotypes were found to be present in our population. Biotype III was most common (25.0%), followed by biotype I (19.6%). A strong correlation was seen between biotypes and *H.*

Table 1. Co-resistance in β -lactamase positive and β -lactamase negative, ampicillin-resistant and ampicillin-sensitive *H. influenzae* isolates

Co-resistance to antimicrobials	Ampicillin sensitive (<i>n</i> = 772)	Ampicillin resistant (<i>n</i> = 229)		
		Total	β -Lactamase positive (<i>n</i> = 196)	β -Lactamase negative (<i>n</i> = 33)
None*	166 (21.5%)	15 (6.5%)	11 (5.6%)	4 (12.1%)
Co-trimoxazole* alone	237 (30.7%)	32 (13.9%)	25 (12.7%)	7 (21.1%)
Erythromycin alone	32 (4.1%)	11 (4.8%)	8 (4.1%)	3 (9.0%)
Chloramphenicol alone	62 (8.0%)	15 (6.5%)	14 (7.1%)	1 (3.0%)
Co-trimoxazole + erythromycin	71 (9.2%)	18 (7.8%)	13 (6.6%)	5 (15.1%)
Co-trimoxazole + chloramphenicol*	133 (17.2%)	65 (28.4%)	55 (28.0%)	10 (30.3%)
Chloramphenicol + erythromycin*	14 (1.8%)	13 (5.7%)	13 (6.6%)	0 (0.0%)
Chloramphenicol + erythromycin + co-trimoxazole*	57 (7.4%)	57 (25.0%)	57 (29.1%)	3 (9.0%)

*The difference between the groups ampicillin sensitive and total ampicillin resistant is significant.

Table 2. Distribution of *H. influenzae* biotypes and serotypes colonizing the nasopharynx of healthy school-going childrenType b, *H. influenzae* type b; CI, confidence interval.

Biotypes	No. (%) of isolates of each biotype	Type b isolates		Ampicillin-resistant isolates	
		No. (%)	95 % CI	No. (%)	95 % CI
I	196 (19.6 %)	110 (56.1 %)	(0.48–0.63)	112 (57.1 %)	(1.09–1.63)
II	168 (16.8 %)	71 (42.3 %)	(0.34–0.50)	64 (38.1 %)	(0.49–0.77)
III	250 (25.0 %)	57 (22.8 %)	(0.56–0.68)	38 (15.2 %)	(0.13–0.24)
IV	94 (9.4 %)	19 (20.1 %)	(0.12–0.29)	5 (5.3 %)	(0.20–0.13)
V	105 (10.5 %)	28 (26.7 %)	(0.18–0.36)	5 (4.8 %)	(0.20–0.12)
VI	51 (5.1 %)	14 (27.5 %)	(0.16–0.41)	4 (7.8 %)	(0.05–0.25)
VII	106 (10.6 %)	17 (16.0 %)	(0.15–0.24)	1 (0.9 %)	(0.01–0.01)
VIII	31 (3.1 %)	0 (0.0 %)	(0–0.11)	0 (0 %)	(0–0.11)
Total	1001	316 (31.6 %)	–	229 (22.9 %)	–

influenzae type b (Table 2). In biotype I *H. influenzae*, type b is more frequent than non-type b, while in the rest of the biotypes non-type b *H. influenzae* is more common than type b. All the biotype VIII isolates were non-type b *H. influenzae*.

Of the 229 ampicillin-resistant *H. influenzae*, 93.4 % (214/229) were biotypes I, II and III, of which 48.9 % were biotype I, 27.9 % were biotype II and 16.6 % were biotype III. Only 6.6 % (15/229) of the strains belonging to biotypes III–VIII were ampicillin resistant. Of the 316 *H. influenzae* type b, 44 % (139/316) were ampicillin resistant, while only 13.1 % (90/685) non-type b *H. influenzae* isolates were ampicillin resistant.

DISCUSSION

This study reports a high nasopharyngeal carriage of *H. influenzae* (41.7 %), especially serotype b (13.2 %) and biotypes I–III (61.3 %). The overall frequency of ampicillin-resistant *H. influenzae* was 22.9 %, of which 85.6 % were β -lactamase producing. Of the isolates, 31.6 % (316/1001) were type b, 44 % of which were ampicillin resistant. A strong correlation between *H. influenzae* biotypes and ampicillin resistance was noted. The majority of ampicillin-resistant isolates are biotype I, forming 49 % of strains. The number of ampicillin-resistant isolates of biotypes II and III is significantly lower as compared to biotype I. Ampicillin resistance is very low amongst biotypes IV–VIII, which also have low pathogenicity. Biotypes I, II and III were significantly more resistant to ampicillin as compared to other biotypes. Co-resistance with co-trimoxazole, erythromycin and chloramphenicol was significantly more associated with β -lactamase-positive organisms.

Reports from different parts of the world describe the carriage rate of *H. influenzae* as ranging from 11.6 to 76 % (Talon *et al.*, 2000; Uraz *et al.*, 2000; Josette *et al.*, 2001; Das

et al., 2002). A study from India has also reported a high *H. influenzae* type b carriage rate (57 %) in healthy school-going Indian children aged 5–10 years (Das *et al.*, 2002). Weather conditions like cold and heavy rainfall, along with the low socio-economic status of the children, may be contributing factors resulting in the high prevalence. Some studies describe the *H. influenzae* type b carriage rate in healthy children to be around 10 %; the carriage rates in children were reported to be 9.6 % in day care centres and 9.1 % in elementary schools in Turkey (Bakir *et al.*, 2002), 7 % in Thailand, and 12 % in Gambia and Venezuela (Olsen *et al.*, 2005; Adegbola *et al.*, 2005; Castillo-Febres *et al.*, 2005). Published studies have reported a high prevalence of ampicillin-resistant *H. influenzae* in India (15.8 %, Ayyagari *et al.*, 1985; 20 %, Patwari *et al.*, 1996; 46 %, IBIS, 2002; 21.1 %, Nag *et al.*, 2001 and 79 %, Das *et al.*, 2002). The PROTEKT study (Kohno *et al.*, 2003) has reported a carriage rate of 64.7 % for ampicillin-resistant *H. influenzae* in South Korea, 17.1 % in Hong Kong and 8.5 % in Japan. A study done in 1994–1995 of 1537 clinical isolates obtained from 30 medical centres in the United States reported the prevalence of ampicillin-resistant β -lactamase-positive *H. influenzae* isolates to be 36.4 % (Doern *et al.*, 1997). Doern *et al.* (1999) reported the prevalence of ampicillin-resistant β -lactamase-producing *H. influenzae* isolates in the United States to be 34.2 % as a part of the SENTRY program. They further commented that the incidence of ampicillin-resistant β -lactamase-producing *H. influenzae* strains in North America levelled off at approximately 30 %. Gordon *et al.* (2003) reported a high prevalence of ampicillin-resistant isolates of *H. influenzae* in North America (27.9 %). The PROTEKT study (a world-wide study) reported the highest prevalence of ampicillin-resistant *H. influenzae* from South Korea (64.7 %) and the lowest from Italy (1.8 %) (Kohno *et al.*, 2003).

Serotype b is the pathogenic *H. influenzae* type and biotype I causes mostly invasive diseases like meningitis in children, while biotypes II and III are more commonly associated with

upper respiratory tract infections like otitis media and conjunctivitis (Kilian, 1976; Gratten, 1983; Alrawi *et al.*, 2002). Das *et al.* (2002) found that the most common biotype was biotype I (36 %), followed by II (18 %) and III (18 %). However, we observed that biotype III was most common (25 %), followed by biotypes I (19.6 %) and II (16.8 %). Another study from North India based on invasive *H. influenzae* isolates reported that biotypes I, II & III were common among both serotype b and non-typable isolates. Biotype I was predominant (40 %) among the typable strains, although most of the non-typable isolates (39 %) were biotype II (Sharma *et al.*, 2002). Teng *et al.* (1989) reported that biotype III has a higher incidence of ampicillin resistance among the different biotypes. Dabernat *et al.* (1988) reported that 50 % of encapsulated-type strains were biotype I, and often resistant to ampicillin (38.5 %), while 42 % of non-encapsulated strains were of biotype II with 10.6 % resistant to ampicillin. Granato *et al.* (1983) also reported that encapsulated strains of biotype I had the highest frequency of ampicillin resistance. Among *H. influenzae* isolates, serotype b strains predominated (83 %), and most (96 %) belonged to biotypes I or II (Gratten *et al.*, 1985). A study of isolates of *H. influenzae* recovered from meningitis (Kilian *et al.*, 1979) reported that 121 (93 %) isolates belonged to biotype I. Kilian (1976) reported that the normal commensal flora of *H. influenzae* from the upper respiratory tract belonged to biotypes II and III. This observation suggests a possible additional virulence factor associated with biotype I organisms. In direct conflict to our findings, Long *et al.* (1983) suggested a tendency for less antibiotic resistance among serotypable versus non-serotypable isolates, and significantly less resistance among biotype I versus other biotype isolates. Albritton *et al.* (1978) reported significantly greater antibiotic resistance among biotype I and II. Gratten *et al.* (1985) reported that the carriage of non-serotypable biotype I and III organisms in healthy and sick children in Papua New Guinea does not differ significantly from that of serotypable biotype I and III strains.

Two mechanisms reported for the development of resistance to β -lactams in *H. influenzae* are (a) the production of β -lactamase, and (b) altered penicillin-binding-protein target sites (Mendelman *et al.*, 1984). The carriage of a high molecular mass conjugative plasmid in *H. influenzae* type b isolates constitutes the genetic basis of resistance to ampicillin, chloramphenicol and tetracycline (Campos *et al.*, 1989; Levy *et al.*, 1993). Campos *et al.* (2003) reported that at a remarkably high level, 24.5 %, *H. influenzae* type f isolates produced β -lactamase, and showed resistance to other antibiotics like co-trimoxazole, chloramphenicol and tetracycline.

A high carriage of *H. influenzae* isolates of type b and certain biotypes (I, II and III) was found in the nasopharynx of healthy school-going children in North India. Ampicillin resistance is significantly more common in biotype I, II and III and serotype b isolates than in isolates of other biotypes and non-serotype b.

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REFERENCES

- Adegbola, R. A., Secka, O., Lahai, G. & 11 other authors (2005). Elimination of *Haemophilus influenzae* type b (Hib) disease from the Gambia after the introduction of routine immunisation with a Hib conjugate vaccine: a prospective study. *Lancet* **366**, 144–150.
- Albritton, W. L., Penner, S., Slaney, L. & Brunton, J. (1978). Biochemical characteristics of *Haemophilus influenzae* in relationship to source of isolation and antibiotic resistance. *J Clin Microbiol* **7**, 519–523.
- Alrawi, A. M., Chern, K. C., Cevallos, V., Lietman, T., Whitcher, J. P., Margolis, T. P. and Cunningham, E. T., Jr (2002). Biotypes and serotypes of *Haemophilus influenzae* ocular isolates. *Br J Ophthalmol* **86**, 276–277.
- Ayyagari, A., Sharma, P., Chakraborti, A. & Agarwal, K. C. (1985). Isolation and detection of *Haemophilus influenzae* from patients of respiratory tract infections and their antibiotic susceptibility patterns in Chandigarh. *Indian J Chest Dis Allied Sci* **27**, 230–235.
- Bakir, M., Yagci, A., Ulger, N., Akbenlioglu, C., Ilki, A., Soyletir, G. & Basaran, M. (2002). Pharyngeal colonization with *Haemophilus influenzae* type b among healthy Turkish infants and children. *Pediatr Int* **44**, 381–386.
- Campos, J., Chanyangam, M., deGroot, R., Smith, A. L., Tenover, F. C. & Reig, R. (1989). Genetic relatedness of antibiotic resistance determinants in multiply resistant *Haemophilus influenzae*. *J Infect Dis* **60**, 810–817.
- Campos, J., Román, F., Pérez-Vázquez, M., Aracil, B., Oteo, J., Cercenado, E. & the Spanish Study Group for *H. influenzae* type f (2003). Antibiotic resistance and clinical significance of *Haemophilus influenzae* type f. *J Antimicrob Chemother* **52**, 961–966.
- Castillo-Febres, O., Lopez, G., Ortega, F., Estopinan, M., Casanova-Escalona, L., Sanchez-Naveda, M., Decker, M. & Edwards, K. M. (2005). Oropharyngeal carriers in Venezuelan children in two child care settings, vaccinated or not with *Haemophilus influenzae* type b tetanus toxoid conjugate vaccine (PRP-T). *Invest Clin* **46**, 15–24.
- Dabernat, H., Delmas, C., Rich, C., Livrelli, V. & Joly, B. (1988). Characterization of *Haemophilus influenzae* isolated from patients with otitis media. *Eur J Clin Microbiol Infect Dis* **7**, 219–225.
- Das, B. K., Arora, N. K., Mathur, P., Ostwal, P., Mandal, S., Kabra, S. K., Kapil, A., Lalitha, M. K. & Thomas, K. (2002). Nasopharyngeal carriage of *Haemophilus influenzae*. *Indian J Pediatr* **69**, 775–777.

- Doern, G. V. (1995). Susceptibility tests of fastidious bacteria. In *Manual of Clinical Microbiology*, 6th edn, pp. 1342–1349. Edited by P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover & R. Tenover. Washington, DC: American Society for Microbiology.
- Doern, G. V., Brueggemann, A. B., Pierce, G., Holley, H. P., Jr & Rauch, A. (1997). Antibiotic resistance among clinical isolates of *Haemophilus influenzae* in the United States in 1994 and 1995 and detection of β -lactamase-positive strains resistant to amoxicillin-clavulanate: results of a national multicenter surveillance study. *Antimicrob Agents Chemother* **41**, 292–297.
- Doern, G. V., Jones, R. N., Pfaller, M. A., Kugler, K. & The SENTRY Participants Group (1999). *Haemophilus influenzae* and *Moraxella catarrhalis* from patients with community-acquired respiratory tract infections: antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United States and Canada, 1997). *Antimicrob Agents Chemother* **43**, 385–389.
- Gordon, K. A., Biedenbach, D. J. & Jones, R. N. (2003). Comparison of *Streptococcus pneumoniae* and *Haemophilus influenzae* susceptibilities from community-acquired respiratory tract infections and hospitalized patients with pneumonia: five-year results for the SENTRY antimicrobial surveillance program. *Diagn Microbiol Infect Dis* **46**, 285–289.
- Granato, P. A., Jurek, E. A. & Weiner, L. B. (1983). Biotypes of *Haemophilus influenzae*: relationship to clinical source of isolation, serotype, and antibiotic susceptibility. *Am J Clin Pathol* **79**, 73–77.
- Gratten, M. (1983). *Haemophilus influenzae*, biotype VII. *J Clin Microbiol* **18**, 1015–1016.
- Gratten, M., Barker, J., Shann, F., Gerega, G., Montgomery, J., Kajoi, M. & Lupiwa, T. (1985). The aetiology of purulent meningitis in highland children: a bacteriological study. *P N G Med J* **28**, 233–240.
- Invasive Bacterial Infection Surveillance (IBIS) Group of the International Clinical Epidemiology Network (2002). Are *Haemophilus influenzae* infections a significant problem in India? A prospective study and review. *Clin Infect Dis* **34**, 949–957.
- Jain, A., Kumar, P. & Awasthi, S. (2005). High nasopharyngeal carriage of drug resistant *Streptococcus pneumoniae* and *Haemophilus influenzae* in North Indian schoolchildren. *Trop Med Int Health* **10**, 234–239.
- Kilian, M. (1976). A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. *J Gen Microbiol* **93**, 9–62.
- Kilian, M., Sørensen, I. & Frederiksen, W. (1979). Biochemical characteristics of 130 recent isolates from *Haemophilus influenzae* meningitis. *J Clin Microbiol* **9**, 409–412.
- Kohno, S., Hoban, D. & The PROTEKT Surveillance Study Group (2003). Comparative *in vitro* activity of telithromycin and β -lactam antimicrobials against bacterial pathogens from community-acquired respiratory tract infections: data from the first year of PROTEKT (1999–2000). *J Chemother* **15**, 335–341.
- Levy, J., Verhaegen, G., Mol, P. D., Couturier, M., Dekegel, D. & Butzler, J. P. (1993). Molecular characterization of resistance plasmids in epidemiologically unrelated strains of multiresistant *Haemophilus influenzae*. *J Infect Dis* **168**, 177–187.
- Long, S. S., Teter, M. J. & Gilligan, P. H. (1983). Biotype of *Haemophilus influenzae*: correlation with virulence and ampicillin resistance. *J Infect Dis* **147**, 800–806.
- Markowitz, S. M. (1980). Isolation of an ampicillin-resistant, non- β -lactamase-producing strain of *Haemophilus influenzae*. *Antimicrob Agents Chemother* **17**, 80–83.
- Mendelman, P. M., Chaffin, D. O., Stull, T. L., Rubens, C. E., Mack, K. D. & Smith, A. L. (1984). Characterization of non- β -lactamase-mediated ampicillin resistance in *Haemophilus influenzae*. *Antimicrob Agents Chemother* **26**, 235–244.
- Nag, V. L., Ayyagari, A., Venkatesh, V., Ghar, M., Yadav, V. & Prasad, K. N. (2001). Drug resistant *Haemophilus influenzae* from respiratory tract infection in a tertiary care hospital in North India. *Indian J Chest Dis Allied Sci* **43**, 13–17.
- National Committee for Clinical Laboratory Standards (NCCLS) (2000). *NCCLS Performance Standard for Antimicrobial Susceptibility Testing*, document M 1000-S13. Wayne, PA: NCCLS.
- Olsen, S. J., Dejsirilert, S., Sangsuk, L., Chunsutiwat, S., Dowell, S. E. (2005). Frequent *Haemophilus influenzae* type b colonization in rural Thailand. *Pediatr Infect Dis J* **24**, 739–742.
- Patwari, A. K., Bisht, S., Srinivasan, A., Deb, M. & Chattopadhyay, D. (1996). Aetiology of pneumonia in hospitalized children. *J Trop Pediatr* **42**, 15–20.
- Pittman, M. (1931). Variation and type specificity in the bacterial species *Haemophilus influenzae*. *J Exp Med* **53**, 471–472.
- Raymond, J., Armand-Lefevre, L., Moulin, F., Dabernat, H., Commeau, A., Gendrel, D. & Berche, P. (2001). Nasopharyngeal colonization by *Haemophilus influenzae* in children living in an orphanage. *Pediatr Infect Dis J* **20**, 779–784.
- Sharma, A., Kaur, R., Ganguly, N. K., Singh, P. D. & Chakraborti, A. (2002). Subtype distribution of *Haemophilus influenzae* isolates from North India. *J Med Microbiol* **51**, 399–404.
- Sottnek, F. O. & Albritton, W. L. (1984). *Haemophilus influenzae* biotype VIII. *J Clin Microbiol* **20**, 815–816.
- Talon, D., Leroy, J., Dupont, M. J., Bertrand, X., Mermet, F., Thouverez, M. & Estavoyer, J. M. (2000). Antibiotic susceptibility and genotypic characterization of *Haemophilus influenzae* strains isolated from nasopharyngeal specimens from children in day-care centers in eastern France. *Clin Microbiol Infect* **6**, 519–524.
- Teng, L. J., Ho, S. W., Lee, G. S. & Leh, K. T. (1989). Serotypes, biotypes, and antimicrobial susceptibility of clinical isolates of *Haemophilus influenzae*. *Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zai* **22**, 68–74.
- Uraz, G., Simsek, H. & Celik, B. (2000). β -lactamase activities and resistance to antibiotics of *Haemophilus influenzae*, *H. parainfluenzae* and *H. aphrophilus* strains identified in throat cultures from children. *Drug Metabol Drug Interact* **16**, 217–228.