

Antibiotic susceptibility of 206 *Haemophilus influenzae* isolates collected from children in central Italy

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Abstract. Susceptibility of 206 *H. influenzae* isolates was evaluated by disk diffusion method for 11 antimicrobial agents. No isolates were found to be resistant to third-generation cephalosporins, amoxicillin+clavulanic acid, gentamicin and ciprofloxacin.

Four untypable isolates (1.9%) were β -lactamase producing ampicillin-resistant; one of these was also resistant to chloramphenicol. The rate of resistance against rifampin was 0.5 percent.

Key words: Antibiotic susceptibility, *Haemophilus influenzae*

Introduction

Following the initial reports of the early 1970s, ampicillin resistance of *Haemophilus influenzae* isolates has been documented worldwide [1–6]. In different geographic areas various prevalences have been observed with a progressive increase in time [1, 2, 4, 7–11]. Apart from the production of β -lactamase (TEM-1c, ROB-1) encoded by plasmid genes, ampicillin resistance can be due to chromosomal mutations responsible for Penicillin Binding Proteins alterations [12–14]. This type of resistance, which is more difficult to detect in the laboratory, is a clinical problem in that it can endanger the successful outcome of therapy based on β -lactamase-resistant drugs [15].

Resistances in *H. influenzae* have been reported for other antibiotics: chloramphenicol, tetracycline, erythromycin, rifampin, and cotrimoxazole. Moreover, some strains have multiple resistance [1, 16].

In the last decade several collaborative studies on *H. influenzae* resistance have been performed in the USA and Europe [2, 8, 11]. In the second European collaborative study [8], seven Italian laboratories took part but the total number of strains presented was very low (205). In Italy, knowledge in this field is fragmentary; in a survey on 327 *H. influenzae* isolates obtained from pediatric and adult patients in northern Italy from January 1984 to December 1989, resistance to ampicillin and chloramphenicol was found to be uncommon [17].

In this paper we report the results of an investigation carried out on the susceptibility of *H. influenzae* isolates collected from pediatric patients and carriers in an area of central Italy.

Collection and identification of isolates

From January 1989 through December 1992, 206 strains of *H. influenzae* were consecutively isolated at the Institute of Infectious Diseases in Siena from 197 children. These included:

- 87 hospitalized children (aged 4 months–14 years, mean age 3.5 years), admitted to the hospital for meningitis, sepsis, lower and upper respiratory tract infections, conjunctivitis;
- 60 outpatients (aged 1–10 years, mean age 5 years), affected by upper respiratory tract infections;
- 51 apparently healthy subjects (aged 6 months–12 years, mean age 4 years), attending the Institute for routine check-ups (physical growth assessment, neurological and psychological development assessment, prevaccinal screenings, baseline laboratory data controls).

Isolation was performed by sample inoculation on chocolate agar plates with bacitracin (Unipath SpA, Italy). Following incubation for 18–24 h at 37 °C in an atmosphere supplemented with 5–7% CO₂, the colonies suspected of *Haemophilus* spp. were assayed for factors X (hemin) and V (NAD), both of which are necessary for *H. influenzae* growth [18].

Serotyping was carried out by the co-agglutination method (karoBio Diagnostic, Sweden). The encapsulated isolates were then typed by counterimmunoelectrophoresis (CIE) using specific antisera for serotypes a–f (Difco Laboratories, Detroit, USA). The biotype was determined according to Slack [18] using the API 10S kit (bioMérieux, Italy).

Production of β -lactamase

All isolates were assayed for β -lactamase production using nitrocefin-impregnated disks (Cefinase, Becton Dickinson, Italy), as suggested by Mendelman et al. [19].

Susceptibility testing

The susceptibility testing of 11 antibiotics was performed using the agar diffusion method of Kirby and Bauer. Owing to the particular growth requirements of *H. influenzae*, we used the Mueller Hinton II chocolate-Agar (5% horse blood) supplied by bioMerieux (Italy) [19]. The antibiotic assay disks supplied by Becton Dickinson (Italy) contained: ampicillin (10 mcg), amoxicillin (20 mcg) + clavulanic acid (10 mcg), cefixime (5 mcg), cefuroxime (30 mcg), cefotaxime (30 mcg), ceftriaxone (30 mcg), chloramphenicol (30 mcg), rifampin (30 mcg), gentamicin (10 mcg), erythromycin (15 mcg), and ciprofloxacin (5 mcg). The susceptibility/resistance, the reliability and reproducibility of the results were based on the recommendations of the National Committee for Clinical Laboratories Standards (NCCLS) [20].

In order to highlight a possible non- β -lactamase-mediated ampicillin resistance, ten untypable isolates which, with 10 mcg ampicillin disks showed, in assays repeated several times, traces of inhibition close to the NCCLS breakpoints of susceptibility/resistance, were assayed also with disks containing 2mcg of ampicillin [21].

Two hundred and six isolates were collected from 197 subjects; in eight subjects two or more isolates from different sites were collected (total 17); these differed with regard to capsule, biotype and susceptibility. Table 1 reports the isolation site of the strains collected: 153 (74.3%) were from the upper respiratory tract (URT) from patients and carriers; other

isolates coming from patients were 29 (14%) from eyes, 3 (1.5%) from sputum, 2 (1%) from ears, 1 (0.5%) from the genitourinary tract, 5 (2.4%) from blood and 13 (6.3%) from cerebrospinal fluid (CSF).

Thirty-two isolates (15.5%) were type b: 100% of blood and CSF isolates, 7.8% of URT isolates and 3.5% of eye isolates belonged to this type (Table 1). Eight URT isolates (3.9%) were encapsulated, but non type b (Table 1): one was type c, five were type e and two type f. One hundred and sixty-six isolates (80.6%) mostly from the URT and the eye were unencapsulated.

The distribution of biotypes according to the isolation sites is shown in Table 1. All blood isolates and 11 out of 13 isolates from CSF belonged to biotype I. The respiratory isolates were, on the other hand, found in all biotypes, but prevalently in biotypes III and I.

All isolates were susceptible to amoxicillin+clavulanic acid, cefixime, cefotaxime, ceftriaxone, gentamicin and ciprofloxacin.

Four untypable isolates (1.9%), three isolates from the URT and one from the eye were β -lactamase-producing and ampicillin resistant (Table 2). Non β -lactamase-producing ampicillin-resistant isolates were not detected.

As to chloramphenicol, three isolates showed susceptibility of intermediate type and one untypable isolate from the URT was resistant (the same isolate was β -lactamase-producing and ampicillin-resistant). One isolate was rifampin-resistant; another isolate showed an intermediate susceptibility. No isolates were susceptible to erythromycin (Table 2), 31.6% were resistant and the remaining showed a susceptibility of intermediate type.

The comparison between these results with those obtained by other studies seems difficult due to different populations controlled and various methods used.

The second European collaborative study [8] reports that 27.1% of the isolates taken from Italian

Table 1. Distribution of *H. influenzae* serotypes and biotypes according to isolation sites

Isolation site	Isolates (n)	Type b	Non type b	Untypable	Biotypes							
					I	II	III	IV	V	VI	VII	VIII
Sputum	3	0	0	3	0	0	3	0	0	0	0	0
URT	153 ^a	12	8 ^b	133	32	28	50	11	4	3	8	17
Eye	29	1	0	28	2	9	15	2	0	0	1	0
Ear	2	1	0	1	2	0	0	0	0	0	0	0
Genitourinary tract	1	0	0	1	0	1	0	0	0	0	0	0
Blood	5	5	0	0	5	0	0	0	0	0	0	0
CFS	13	13	0	0	11	0	0	2	0	0	0	0
Total	206	32 (15.5%)	8 (3.9%)	166 (80.6%)	52	38	68	15	4	3	9	17

^a Isolates from patients and carrier.

^b 5 type e, 2 type f, 1 type c.

Table 2. Antibiotic susceptibility of 206 strains of *H. influenzae*

<i>H. influenzae</i>	Number of isolates	AM			C			RA			E		
		S	I	R	S	I	R	S	I	R	S	I	R
Type b	32	32			32			32				31	1
Non type b	8	8			8			8				4	4
Untypable	166	162		4	162	3	1	164	1	1		106	60
Total	206	202		4 (1.9%)	202	3	1 (0.5%)	204	1	1 (0.5%)		141	65 (31.6%)

AM = ampicillin; C = chloramphenicol; RA = rifampin; E = erythromycin.
S = susceptible; I = intermediate; R = resistant.

laboratories were type b. The prevalence obtained in our survey, carried out in a limited geographic area of our country (southern Tuscany), is lower (15.5%), but quite similar to that reported by a study performed in another area of Italy [17].

The URT isolates were prevalently unencapsulated. However, their role as pathogens, especially in subjects with predisposing factors, is currently reported [22].

In agreement with the literature [18], nearly all isolates coming from invasive infections were biotype I. Most of the isolates from carriers and localized infections were biotypes III, II, VIII and IV.

Ampicillin susceptibility was satisfactory; only four unencapsulated isolates (1.9%) showed β -lactamase-mediated ampicillin-resistance. Noteworthy, even when using the disks containing 2 mcg as suggested by Mendelman [21] and Powell et al. [15], non β -lactamase-mediated ampicillin resistance was not observed. This type of resistance has been reported in the USA, Canada and New Zealand, and also in the UK and the Netherlands [23, 24]. The difficulty in detecting it in clinical laboratories is still a problem [25].

Different prevalences of ampicillin resistance have been reported in various European countries [8]. The results obtained in our geographic area, as well as in other Italian areas [17], are similar to those obtained in Germany, Switzerland and the Netherlands; while they differ profoundly from the results obtained in Belgium, France and especially in Spain [8].

An unencapsulated isolate from the respiratory tract showed a multiple resistance to ampicillin and chloramphenicol. The resistance of unencapsulated *H. influenzae* is not to be underestimated because of the ever growing pathogenic role of this microorganism, and also because only antibiotic therapy is possible due to the lack of a vaccine for untypable strains [26–28].

Rifampin resistance, as reported in the USA, was rare (0.5% of unencapsulated isolates) and erythromycin proved to be, once again, an inactive antibiotic for *H. influenzae* [29]. Third generation cephalosporins tested were active. These drugs, capable of pene-

trating the blood-brain barrier, are currently preferred as therapy for invasive infections from type b *H. influenzae*.

The results can be considered favourable. In our geographic area the phenomenon of *H. influenzae* susceptibility does not appear relevant and the use of traditional drugs, ampicillin and chloramphenicol, for invasive infections is still justifiable.

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