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## *Haemophilus influenzae* drug resistance in France from 2017 to 2021: consideration for treatment of otitis media



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#### ABSTRACT

*Objectives: Haemophilus influenzae* is a prevalent agent of respiratory infections, including acute otitis media (AOM), that lead to high antibiotic prescription and may contribute to the development of bacterial resistance to antibiotics. The objective of this work was to describe and analyse antibiotic resistance of *H. influenzae* from 2017 to 2021 in France.

*Methods:* We characterized *H. influenzae* isolates transmitted to the French national reference centre for *H. influenzae* between 2017 and 2021. We included all the 608 non-invasive respiratory isolates. Resistance rates to the main antibiotics were described. The relationship between resistance rate, age, and sex of patients and germ serotype was investigated.

*Results:* Isolates were mainly from alveolar lavage (29.3%), expectoration (22.9%), or sputum (15%). Resistance to amoxicillin (61.4%), amoxicillin/clavulanic acid (47.4%), and cefotaxime (39.3%) was high and correlated with the presence of  $\beta$ -lactamase and/or modifications of the *ftsI* gene encoding penicillinbinding protein 3. Resistance to sulfamethoxazole/trimethoprim (33.2%) was more moderate. There were no significant differences according to serotype, age, or gender.

*Conclusions:* The benefit/risk balance of first choice use of amoxicillin and even of amoxicillin/clavulanic acid in AOM is questionable in view of the significant resistance to *H. influenzae*. The use of sulfamethox-azole/trimethoprim could be an alternative but may still need further evaluation.

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#### 1. Introduction

Despite a decline in recent years, acute otitis media (AOM) remains one of the most prevalent infections in children worldwide [1–3]. Management of AOM is a primary care issue. In 80% of cases, symptoms of AOM resolve without treatment within a few days [4]. When biological samples are collected, a bacterial origin is detected in only 24% of cases [5]. International guidelines recommend postponing antibiotics and reserving them only for children who are unwell or who are at risk of complications [6,7]. Despite this, AOM, as well as respiratory infections, remain the cause of a large volume of antibiotic prescriptions that are sometimes considered inappropriate [8,9]. This high volume of antibiotic prescriptions is a major issue because it contributes to the worrying in-

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crease in bacterial resistance to antibiotics [10]. In France, in 2020, despite a regular decrease over the last 10 years, the French prescription of  $\beta$ -lactams in outpatient settings remained high with 10.1 daily defined doses per 1,000 inhabitants compared to 6.5 in Europe [11].

According to international guidelines, antibiotic treatment of AOM is empiric and is based on the bacterial epidemiology. In recent decades, *Streptococcus pneumoniae* (30%), *Haemophilus influenzae* (23%), and *Moraxella catarrhalis* (5%) have been reported as the bacterial pathogens most frequently causing AOM [12]. However, the proportion of *H. influenzae* infections is increasing and has become the dominant bacterial cause in AOM in recent years [13–15]. This change could be explained by the increase in vaccination against *S. pneumoniae* [16,17].

Antibiotic treatment varies from country to country but remains empirical, using  $\beta$ -lactams as a first-line treatment. In European countries, first-line antibiotic therapy, when indicated, is based on amoxicillin, with a recourse to amoxicillin/clavulanic acid combi-

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nation if the fever persists for 48-72 h [18]. Some risk factors (but with odd ratios not exceeding 1.3) for H. influenzae AOM are identified (association of otitis, conjunctivitis, winter period, bilateral otitis, >3 previous AOM episodes, and antibiotic consumption in the previous month) [19]. There is no consensus on the use of first line amoxicillin/clavulanic acid combination in cases of risk factors for H. influenzae AOM. Because of the risk of H. influen*zae* resistance to amoxicillin alone by secretion of  $\beta$ -lactamase, in cases of risk factors of H. influenzae AOM, amoxicillin/clavulanic acid combination is recommended in France and the United States but not in the United Kingdom [20-22]. However, these guidelines don't consider mechanisms of resistance to amoxicillin and to amoxicillin/clavulanic acid combination in  $\beta$ -lactamase negative H. influenzae because of increasingly reported mutations of the ftsI gene encoding penicillin binding protein 3 (PBP3) [23]. International guidelines are based primarily on the study of resistance patterns in S. pneumoniae, as there is a lack of data on H. influenzae. The main studies found a resistance rate of 77.3% to amoxicillin and 3.8% to amoxicillin/clavulanic acid combination on samples taken from paracentesis [5,24].

As a response to increasing incidences of antibiotic resistance (in particular, to  $\beta$ -lactams), we aimed in this work to describe the bacterial resistance of *H. influenzae* to the main antibiotics between 2017 and 2021 to provide data that can help foster better use of antibiotics in treating *H. influenzae* infections. The secondary objective of this work was to analyse the determinants of these resistances according to the characteristics of the patients and the *H. influenzae* isolates.

#### 2. Materials and methods

#### 2.1. Data and type of the study

This is a retrospective study based on the French National Reference Center (NRC) database for meningococcus and H. influenzae (NRCMHi). The NRCMHi is a laboratory designated under the authority of the Ministry of Health for the microbiological surveillance of meningococcal and H. influenzae infections [25]. As part of its mission, the NRCMHi receives, characterizes, analyses, and archives the results of H. influenzae isolates. All French medical laboratories and hospitals can send isolates to the NRCMHi. These isolates come from various situations: epidemiological surveillance or confirmation of bacteriological identification in hospital laboratories. For each isolate, the NRCMHi database provided information on the date of sampling, the invasive or non-invasive nature of the sample, the sampling site, the age, and the sex of the patient. The analyses of the isolates performed by the NRCMHi included the serotype (typeable, with identification of the serotype, or nontypeable) and the minimum inhibitory concentration (MIC) to  $\beta$ -lactam antibiotics (ampicillin, amoxicillin, amoxicillin/clavulanic acid combination, and cefotaxim), as well as to other antibiotics (ciprofloxacin, rifampicin, and sulfamethoxazole/trimethoprim).

This work included *H. influenzae* isolates from the NRCMHi database between 2017 and 2021 of all ages [26]. In order to keep the comparison with *H. influenzae* AOM, invasive related isolates and isolates taken outside the respiratory mucosa were not included [23,27,28]. Invasive isolates were those recovered by culture from normally sterile sites.

Data were expressed as absolute numbers and percentages. Means were expressed with their 95% confidence intervals (CIs). Medians were expressed with their interquartile ranges.

In a second step, the resistance profiles of the isolates were compared according to age, sex, and serotypes. We used the 12-year cut-off point frequently used in international guidelines and other studies to separate children from the rest of the population [29,30]. Statistical analyses were performed using the  $\chi^2$  test and

the one-sided Fisher's exact test. A Bonferroni correction was applied by adjusting the  $\alpha$  risk to the number of comparisons performed according to the formula  $\alpha = 0.05/n$  where n is the number of comparisons performed.

#### 2.2. Statistical analysis

Statistical analyses were performed on STATA® software. No legal deposit statement or ethics committee was required.

#### 2.3. Antimicrobial susceptibility testing

The antimicrobial susceptibility test was performed in the NR-CMHi by gradient tests (Etest, bioMérieux, Marcy-l'Etoile, France).  $\beta$ -lactamase production was measured using the cefinase disc method (BioMérieux, France). The European Committee on Antimicrobial Susceptibility Testing guidelines for the antimicrobial susceptibility breakpoints of the MICs of antibiotics were adopted as criteria for interpreting drug susceptibility.

#### 2.4. Molecular analysis of ftsI genes

The sequences of a 621-bp fragment of the ftsl gene (encoding the PBP3), corresponding to nucleotides 977-1597 relative to the ftsl start codon, was obtained by polymerase chain reaction (PCR) using the primers fstI1F 5'gttttcccagtcacgacgttgtaGTTAATGCGTAACCGTGCAATTAC-3' and ftsl1R 5'-ttgtgagcggataacaatttc ACCACTAATGCATAACGAGGATC-3'. DNA sequencing was performed on PCR products using the universal forward and reverse primers (in lower cases of ftsI1F and ftsI1R, respectively). DNA sequences were used to assign alleles for the corresponding isolates through the PubMLST database and analysed through the tools available on the BIGSdb platform. Sequences were aligned using Multiple Sequence Alignment by CLUSTALW and sequences differing by at least one nucleotide were assigned a unique ftsI allele sequence number. Phylogenetic networks were generated using SplitsTree (version 4.14.6) (www.splitstree.org) with default parameters.

#### 3. Results and discussion

#### 3.1. Description of isolates received between 2017 and 2021

Of 1519 *H. influenzae* isolates received by the NRCMHi between 2017 and 2021, a total of 608 isolates from respiratory sites were included in this work. Fig. 1 shows the flow chart of the isolate selection.

The sampling sites of the study included both upper and lower respiratory sites that accounted for 53% and 47%, respectively. The most frequent site was bronchial aspiration (29.28%) followed by expectoration (22.86%) and sputum (14.97%). Table 1 describes the sampling sites of the isolates included in this work.

The mean age of the patients who were sampled was 51.24 y 95% CI (49.03; 53.45), and the median age was 60.74 y (35.76; 71.44). There were 251 women (41.28%) and 357 men (58.72%). Six hundred isolates (98.68%) were non-typeable and 8 (1.32%) were typeable.

The mean age of the child population (under 12 y) was 2.49 y 95% CI (1.89; 3.11). There were 51 (44.74%) boys and 63 (55.26%) girls. One hundred and thirteen isolates (99.12%) were non-typeable and 1 (0.88%) was typeable.

## 3.2. Description of $\beta$ -lactams resistance profiles of the included isolates

The overall level of  $\beta$ -lactam resistance was high, especially for ampicillin, amoxicillin, and amoxicillin/clavulanic acid combina-



Table 1

Collection sites of included isolates $(n = 608)$ .						
Upper respiratory sites						
	n	%				
Expectoration	139	22.86				
Sputum	91	14.97				
Ear	16	2.63				
Rhinopharynx	11	1.81				
Endonasal swab	9	1.48				
Sinus	5	0.82				
Throat	3	0.49				
Mouth swabbing	2	0.33				
Lower respiratory sites						
	n	%				
Bronchial aspiration	178	29.28				
Alveolar lavage	34	5.59				
Tracheal swab	32	5.26				
Protected bronchial sample	30	4.93				
Bronchial fibroscopy	9	1.48				
Alveolar brushing	3	0.49				
Lung biopsy	1	0.16				
<u>Other</u>						
Eye	45	7.40				

tion that were detected in 62.52%, 61.41%, and 47.15% of the tested isolates, respectively. It is noteworthy that resistance to a thirdgeneration cephalosporin, cefotaxim, was also detected at a high level (39.3%). We detected 157  $\beta$ -lactamase positive ampicillinresistant isolates (BLPAR) and 208  $\beta$ -lactamase negative ampicillinresistant (BLNAR) isolates (27.07% and 34.96%, respectively, of all isolates) that accounted for 43.01% and 56.99% of ampicillinresistant isolates, respectively. The percentage of BLNAR increased from 25.30% in 2017 to 40.24% in 2021 (P = 0.02) while the percentage of BLPAR isolates remained stable at 30.12% in 2017 and 20% in 2021 (P = 0.62). The antibiotic resistance profile is summaJournal of Global Antimicrobial Resistance 31 (2022) 222-227

#### Table 2

Antibiotic resistance profiles of Haemophilus influenzae isolates between 2017 and 2021 (n = 608).

Ampicillin							
Susceptible	223 (37.48%)						
Resistant	372 (62.52%)						
Missing data <sup>a</sup>	13						
Amoxicillin							
Susceptible	230 (38.59%)						
Resistant	366 (61.41%)						
Missing data <sup>a</sup>	12						
Amoxicillin/clavulanic acid							
Susceptible	315 (52.85%)						
Resistant	281 (47.15%)						
Missing data <sup>a</sup>	12						
Cefotaxim							
Susceptible	364 (60.67%)						
Resistant	236 (39.33%)						
Missing data <sup>a</sup>	8						
Ciprofloxacin							
Susceptible	546 (91.76%)						
Resistant	49 (8.24%)						
Missing data <sup>a</sup>	13						
Rifampicin							
Susceptible	598 (99.67%)						
Resistant	2 (0.33%)						
Missing data <sup>a</sup>	8						
Sulfamethoxazole	/trimethoprim						
Susceptible	398 (66.78%)						
Resistant	198 (33.22%)						
Missing data <sup>a</sup>	12						
$\beta$ -lactamase							
Positive	157 (27.07%)						
Negative	423 (72.93%)						
Missing data <sup>a</sup>	28						

<sup>a</sup> Missing data correspond to isolates that were not recovered by culture, were massively contaminated, or not performed. The identification of *H. influenzae* was performed using molecular approaches according to Deghmane et al. [23]. Critical data for resistance were MIC >1 mg/L (ampicillin), >2 mg/L (amoxicillin), >0.125 mg/L (cefotaxim), >0.06 mg/L (ciprofloxacin), >1 mg/L rifampicin, and >1 mg/L (Sulfamethoxazole/ trimethoprim).

rized in Table 2. The percentage of resistance to these antibiotics was not significantly different between the period of 2017 to 2019 and the period of 2020 to 2021 (before and since the emergence of COVID-19, respectively).

#### 3.3. Molecular characterization of the resistance to $\beta$ -lactams

The phenotype BLNAR is conferred by modifications of PBP3 that reduce affinity to amoxicillin and ampicillin and several of these modifications in PBP3 also confer resistance to cefotaxime [23]. Therefore, we DNA sequenced the *ftsl* fragment that harbours these modifications from 138 isolates (23% of all the isolates), of which 107 isolates were  $\beta$ -lactamase-producing isolates. We identified 57 ftsl alleles corresponding to 36 different aminoacid sequences. We aligned the deduced amino acid sequences of these 57 ftsI alleles and analyzed the phylogenetic relationships and drew the corresponding tree using SplitsTree4 (Fig. 2). The sequences seem to be clustered into four groups according to the presence/absence of mutations correlated with resistance to ampicillin and/or cefotaxime [23]. Alleles of groups 1 and 2 lack critical mutations but alleles of group 2 show few other polymorphic sites. These alleles were reported to be correlated with susceptibility to ampicillin and cefotaxime [23]. They were represented by 37 iso-



**Fig. 2.** Phylogenetic tree of *fts1* based on the sequence multiple alignment of amino-acid sequences deduced from the DNA sequences of all *fts1* alleles defined among the 107 ß-lactamase negative isolates. The tree was visualized by SplitsTree4, as described in the 'materials and methods' section. The 4 *fts1* groups were according to Deghmane et al. [23] with individual alleles in each group that were indicated by different colours. The grey boxes indicate each group and the geometric means of minimum inhibitory concentrations (MICs) of the isolates of each group of ampicillin and cefotaxime, and the 95% confidence intervals of geometric means are in brackets.

lates among the 107 isolates (35%). Indeed, all the 37 corresponding isolates were susceptible to ampicillin and cefotaxime, with MICs ranging between 0.064 mg/L and 1 mg/L (geometric mean of 0.31 mg/L and 95% CI [0.26–0.37]).

Alleles of group 3 have at least the critical mutations R517H or N526K in addition to other polymorphic sites. These alleles were reported to be correlated with moderate resistance to ampicillin but with susceptibility to cefotaxim [23]. They were represented by 30 isolates among the 107 isolates (28%). Most of the corresponding isolates were resistant to ampicillin (18/30) but susceptible to cefotaxim (26/30), with MICs of ampicillin ranging between 0.190 mg/L and 256 mg/L (geometric mean of 1.8 mg/L and 95% CI [1.1–3.0]) (Fig. 2).

Alleles of group 4 have the critical mutations S357N, M377I, and S385T in addition to the mutations of group 3, R517H or N526K. These alleles were reported to be correlated with moderate resistance to ampicillin and cefotaxim [23]. They were represented by 40 isolates among the 107 isolates (37%). Indeed, 33 of 40 of the corresponding isolates were resistant to ampicillin and 38 of 40 to cefotaxime, with MICs for ampicillin ranging between 0.5 mg/L and 256 mg/L (geometric mean of 5.1 mg/L and 95% CI [2.9–9.2]). MIC for cefotaxim ranged between 0.125 mg/L and 3 mg/L with a geometric mean of 0.58 mg/L and 95% CI (0.45–0.75) (Fig. 2).

The most frequent *fts1* alleles were *fts110* and *fts126*, both represented by 11 isolates (8% of each allele), and they belonged to groups 1 and 4, respectively.

Molecular characteristics of *fts1* and phenotypic determination of MICs for ampicillin showed 82% of observed agreement and a Kappa coefficient of 0.65 (between 0.61–0.80), indicating a good agreement. The agreement was higher for cefotaxime, with 92% of observed agreement and a Kappa coefficient of 0.82, indicating a very good agreement (>0.80).

## 3.4. Description of resistance profiles of the included isolates to other antibiotics

The level of susceptibility to broad-spectrum antibiotics (rifampicin and ciprofloxacin) was high and reached at least 90%. The antibiotic resistance profile is summarized in Table 2. The level of resistance of *H. influenzae* isolates to the sulfamethoxazole/trimethoprim combination was (33.2%) and, therefore, lower than that to ampicillin and amoxicillin and even amoxicillin/clavulanic acid combination. Moreover, 61.83% and 62.84% of ampicillin and amoxicillin resistant isolates, respectively, were still susceptible to sulfamethoxazole/trimethoprim combination.

## 3.5. Relationship between antibiotic resistance patterns and characteristics

Isolates characteristics were compared according to age, sex, and serotype for all tested antibiotics (Table 3). After Bonferroni correction, there was no statistically significant difference in antibiotic resistance profile between children under 12 y of age and those  $\geq 12$  y, or between women and men. Typeable isolates were significantly more susceptible to  $\beta$ -lactams. A non-significant trend existed for other antibiotics.

#### 3.6. Discussion

The study of non-invasive respiratory isolates of *H. influenzae* received by the NRCMHi between 2017 and 2021 found a high level of resistance to ampicillin, amoxicillin, and to the amoxicillin/clavulanic acid combination. We used these isolates as a proxy to evaluate this resistance in isolates responsible for AOM. Indeed, H. influenzae isolates from respiratory pathways were reported to be similar and have been involved in sinusitis, AOM, respiratory tract infections, and conjunctivitis [27,31]. Resistance to amoxicillin was close to the other work in the literature on the subject (77.3% vs. 61.40% in this work) [32]. Resistance to the amoxicillin/clavulanic acid combination was, on the other hand, significantly higher than that identified so far (3.8% vs. 47.20% in our work) [5]. Haemophilus influenzae resistance mechanisms are conferred either by  $\beta$ -lactamase secretion (BLPAR) or by modification of PBP3 that reduces affinity to amoxicillin and ampicillin (BLNAR) or by both [33]. Some of these modifications in PBP3 also confer resistance to cefotaxim [23]. The difference may be due to the high level of modified PBP3 in French data (26% expression of altered PBP3 in 2018) [34] compared to the other work [24], as also suggested by our work (25.30% in 2017 to 40.24% in 2021).

#### Table 3

Relationship between antibiotic resistance profile and isolate characteristics for  $\beta$ -lactams (n = 608).

		Age		Sex		Serotype	
		Child <12 y	Adult $\geq 12y$	Women	Men	Non typeable	Typeable
Ampicillin	Susceptible	50 (35.74%)	173 (35.74%)	89 (36.18%)	134 (38.40%)	216 (36.80%)	7 (87.50%)
	Resistant	61 (64.26%)	311 (64.26%)	157 (63.88%)	215 (61.60%)	371 (63.20%)	1 (12.50%)
	Р	$P = 0.07^{a}$		$P = 0.58^{a}$		$P = 0.005^{a}$	
Amoxicillin	Susceptible	48 (43.24%)	182 (37.53%)	92 (37.25%)	138 (39.54%)	223 (37.93%)	7 (87.50%)
	Resistant	63 (56.76%)	303 (62.47%)	155 (62.75%)	211 (60.46%)	365 (62.07%)	1 (12.50%)
	Р	$P = 0.26^{a}$		$P = 0.57^{a}$		$P = 0.006^{a}$	
Amoxicillin	Susceptible	63 (56.76%)	252 (51.96%)	126 (51.01%)	189 (54.15%)	307 (52.21%)	8 (100%)
Clavulanic acid	Resistant	48 (43.24%)	233 (48.04%)	121 (48.99%)	160 (45.85%)	281 (47.79%)	0 (0%)
	Р	P=0.36 <sup>a</sup>		$P = 0.45^{a}$		$P = 0.006^{a}$	
Cefotaxim	Susceptible	79 (71.17%)	285 (58.28%)	151 (61.13%)	213 (60.34%)	357 (39.70%)	7 (87.50%)
	Resistant	32 (28.83%)	204 (41.72%)	96 (38.87%)	140 (39.66%)	235 (39.70%)	1 (12.50%)
	Р	$P = 0.01^{a}$		$P = 0.85^{a}$		$P=0.11^{a}$	
Ciprofloxacin	Susceptible	105 (94.59%)	441 (91.12%)	221 (89.84%)	325 (93.12%)	538 (91.65%)	8 (100%)
	Resistant	6 (5.41%)	43 (8.88%)	25 (10.16%)	24 (6.88%)	49(8.35%)	0 (0%)
	Р	P=0.23 <sup>a</sup>		P=0.15 <sup>a</sup>		$P = 0.5^{a}$	
Rifampicin	Susceptible	111 (100%)	487 (99.59%)	246 (99.60%)	352 (99.72%)	590 (99.66%)	8 (100%)
	Resistant	0 (0%)	2 (0.41%)	1 (0.4%)	1 (0.28%)	2 (0.34%)	0 (0%)
	Р	N/A		$P = 1.00^{b}$		N/A	
Sulfamethoxazole	Susceptible	79 (71.17%)	319 (65.77%)	159 (64.37%)	239 (64.48%)	390 (66.33%)	8 (100%)
Trimethoprim	Resistant	32 (28.83%)	166 (34.23%)	88 (35.63%)	110 (31.52%)	198 (33.67%)	0 (0%)
	Р	P=0.28 <sup>a</sup>		P=0.29 <sup>a</sup>		N/A	

 $a \chi^2$  test.

<sup>b</sup> Fisher's exact test.

Resistance to rifampicin and ciprofloxacin was rather low. However, their broad spectrum of action may increase selection of antibiotic resistance; therefore, these options are not acceptable for widespread use in AOM.

Resistance to sulfamethoxazole/trimethoprim was lower than that to amoxicillin or to amoxicillin/clavulanic acid combination. The sulfamethoxazole/trimethoprim combination is already indicated as a second-line therapy in cases of  $\beta$ -lactam allergy. Moreover, most isolates were non-typeable and vaccination would have no further effect, as vaccination against *H. influenzae type b* isolates has reduced their proportion in AOM. Vaccination research against non-typeable isolates might be an unmet medical need.

Our data on molecular analysis of *fts1* confirm the reliability of using this approach to predict susceptibility/resistance to  $\beta$ -lactams with a good agreement with phenotypic antibiogram. Moreover, our data further show the high heterogeneity among  $\beta$ lactam-resistant isolates that was reflected by the high number of modified *fts1* alleles that harboured critical mutations in *fts1*.

This work was carried out over five years and allowed for the collection of many isolates from all over France, reinforcing the generalization of its results. Moreover, the emergence of COVID-19 does not seem to be associated with changes in sensitivity profiles. However, the origin of these isolates was not limited to the ear but the whole respiratory mucosa and did not concern only children. Nevertheless, the fact that *H. influenzae* originates from the respiratory mucosa in AOM [27] and the absence of a statistically significant difference between children and adults in this work lead authors to consider that these results may be extrapolated to the AOM situation.

The data from this work confirm the value of delaying antibiotic therapy or reserving it only for unwell children or those at risk of complications [7]. Considering the resistance observed in this work and the now dominant position of *H. influenzae* in the AOM bacterial epidemiology, the benefit-risk ratio of the use of amoxicillin and amoxicillin/clavulanic acid combination seems less relevant than it was, especially in France. Given the high volume of prescriptions for AOM, the risk of selection of resistant germs has increased.

The choice of using the amoxicillin/clavulanic acid combination as the first-line antibiotic in France in cases of suspected *H. influenzae* AOM should be strongly reconsidered [6]. The use of sulfamethoxazole/trimethoprim could be an alternative if there are risk factors for *H. influenzae*. This work also questions the use of amoxicillin in the antibiotic strategy for AOM. Such reflection on antibiotic choices should consider the resistance profiles of other germs involved in AOM (especially *S. pneumoniae*), the potential for selection of resistant germs, and the adverse effects of other available antibiotics. Given the susceptibility of *S. pneumoniae*, the option of a sulfamethoxazole/trimethoprim combination might be the subject of further work [5]. Further work is also needed on the antibiotic strategy for other respiratory diseases, such as chronic bronchitis and sinusitis, where non-typeable *H. influenzae* is frequently involved.

The progression of *H. influenzae* AOM and its resistance profile to first-line antibiotics used in AOM may lead to reconsideration of the use of amoxicillin and even amoxicillin/clavulanic acid combination in first-line treatment, even in AOM associated with risk factors of *H. influenzae* AOM.

The susceptibility of *H. influenzae* to sulfamethoxazole/trimethoprim shared by *S. pneumoniae* may be considered as an alternative first-line treatment for AOM.

**Availability of data and materials:** Anonymized individual data that support the findings of this study are available upon request.

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#### **Ethical approval**

Haemophilus influenzae isolates are sent to the National Reference Centres for meningococci and Haemophilus influenzae (NR-CMHi) as part of its mission in the national surveillance that is approved by the Ministry of Health. Procedures for collecting samples and information were submitted and approved by the CNIL N°1475242/2011 (Commission Nationale de l'Informatique et des Libertés). Metadata on the cases are anonymised in the database of NRCMHi using an irreversible double encryption with no information allowing identification of patients.

#### **Competing interests**

None declared

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