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β-Lactam resistance among *Haemophilus influenzae* isolates in Poland

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ABSTRACT

Objectives: Haemophilus influenzae is a human-specific Gram-negative coccobacillus responsible for a significant number of respiratory tract infections and severe invasive infections such as meningitis and sepsis. The purpose of this study was to characterise the mechanisms of β-lactam resistance among Polish *H. influenzae* isolates and to evaluate the resistance detection methods applied.
Methods: This study was conducted on 117 Polish *H. influenzae* isolates collected in 2012. Minimum inhibitory concentrations were assessed by broth microdilution. All strains were evaluated using the disk diffusion method and the algorithm proposed by the Nordic Committee on Antimicrobial Susceptibility Testing (NordicAST). To detect changes in penicillin-binding protein 3 (PBP3), PCR screening was performed, followed by *ftsI* gene sequencing.
Results: Neither β-lactamase production nor PBP3 alterations were demonstrated in 76 isolates (65.0%). Susceptibility to ampicillin, amoxicillin, amoxicillin/clavulanic acid, cefuroxime (intravenous) and ceftriaxone was observed in 70.9%, 78.6%, 98.3%, 82.9% and 100% of the isolates, respectively. β-Lactamase production characterised 21 isolates (17.9%). Screening PCR identified 20 isolates (17.1%) with PBP3 alterations, and according to subsequent *ftsI* sequencing all these strains were finally recognised as gBLNAR (genetically β-lactamase-negative, ampicillin-resistant), among which 65.0% were ampicillin-resistant. According to molecular classification of PBP3 alterations, 95.0% of gBLNAR belonged to group II, representing four subgroups IIa–IId.
Conclusions: Haemophilus influenzae resistance to antibiotics requires continuous attention, effective detection methods and a rational policy of antibiotic usage. The algorithm proposed by NordicAST can be applied in routine laboratory work, whereas sequencing of the *ftsI* gene may be useful in molecular epidemiology studies.

1. Introduction

Haemophilus influenzae is a human-specific Gram-negative coccobacillus commonly found in the upper respiratory tract as part of the physiological bacterial flora. It may also be responsible for a significant number of respiratory tract infections as well as severe invasive infections such as meningitis and sepsis, both in children and in adults.

For many years infections caused by *H. influenzae* were treated successfully with ampicillin. However, during recent decades resistance to this antibiotic as well as to other β-lactams has become more common. Two major resistance mechanisms have been described, namely enzymatic hydrolysis by β-lactamases and alterations in penicillin-binding protein (PBP). Traditionally, the most commonly occurring mechanism of resistance to β-lactams in *H. influenzae* was β-lactamase production. β-Lactamase-mediated resistance to ampicillin was described in the early 1970s [1]. The term BLPAR (β-lactamase-positive, ampicillin-resistant) has become a frequently used acronym for strains carrying such a mechanism. *Haemophilus influenzae* strains proven to produce plasmid-mediated β-lactamases, specific enzymes (TEM, ROB and VAT) similar to bacterial transpeptidases in their structure, are able to effectively neutralise target antibiotics [2].

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Five PBPs (1A, 1B, 2, 3 and 4) are known in *H. influenzae*. Alterations in one of them, namely PBP3 encoded by the *ftsI* gene, have been attributed to increased resistance to β-lactam antibiotics [3,4]. Several mutations within the transpeptidase domain of PBP3 have been recognised, some of which result in decreased affinity for penicillins as well as cephalosporins. Strains carrying altered PBPs have been named BLNAR (β-lactamase-negative, ampicillin-resistant) since the 1970s [5–7]. BLNAR isolates are characterised by the presence of either N526K or R517H substitution in PBP3, encoded by the altered *ftsI* gene [8]. Several classifications have been proposed based on PBP3 amino acid substitutions [5,6,9–11]. Those widely accepted are the classifications by Ubukata et al. [5] and Dabernat et al. [10]. The former divided *H. influenzae* strains into three groups. Isolates with the R517H substitution were designated to group I, whilst N526K combined with some other substitution(s) were designated to group II. Finally, group III is defined based on N526K mutation combined with M377I, S385T or L389F substitution [5]. Dabernat et al. further subdivided group II [10]. Although the prevalence of BLNAR strains varies considerably between different countries, a significant increase has been noted in many European countries and throughout the world over the last years [12–14]. Moreover, certain *H. influenzae* strains carry alterations in PBP and concomitantly are able to produce β-lactamases (BLPACR; β-lactamase-positive, amoxicillin/clavulanic acid-resistant).

Despite this, some authors have pointed out the role of other mechanisms [15]. Disrupted repression of the AcrR efflux pump and alterations in outer membrane protein 2 (OMP2) are widely discussed, with contradictory results being obtained [3,9,16]. The clinical relevance of these findings remains unclear, and the main focus of research concentrates on the classical mechanisms mentioned above.

The aim of this study was to analyse mechanisms of resistance expressed by *H. influenzae* strains isolated in Poland, to describe their susceptibility to selected β-lactam antibiotics and to evaluate different methods of laboratory detection of resistance to β-lactams in *H. influenzae*.

2. Materials and methods

2.1. Study isolates

This study was conducted on 117 *H. influenzae* Polish isolates collected at the National Reference Center for Bacterial Meningitis (NRCBM), Department of Epidemiology and Clinical Microbiology of the National Medicines Institute in Warsaw, Poland, between January–December 2012. All strains included in the study were obtained within two continuous surveillance programmes conducted by the NRCBM. The first surveillance programme consisted of isolates ($n = 89$) responsible for community-acquired lower respiratory tract (LRT) infections [17]. The second source comprised isolates from the surveillance programme of community-acquired invasive bacterial infections in Poland.

A total of 28 strains (23.9%) were isolated from cerebrospinal fluid and blood and the remaining 89 (76.1%) were obtained from the LRT (sputum, bronchoalveolar lavage and endoscopic aspirate).

All strains were re-identified phenotypically according to standard laboratory procedures [18]. In addition, PCR was performed to confirm species, for serotype identification and to detect capsule-specific genes [19,20].

2.2. Strain denomination according to screening PCR and β-lactamase production

Screening PCR was carried out for all isolates to detect mutations in the *ftsI* gene associated with N526K and/or S385T substitution, using the H-PBP3-S and H-PBP3-BLN single nucleotide polymorphism-based primer sets described by Hasegawa et al. [21]. H-PBP3-S primers were designed to recognise β-lactamase-negative, ampicillin-susceptible (BLNAS) strains with no pre-defined changes in PBP3 (no N526K substitution), whilst H-PBP3-BLN primers were designed to identify strains with both N526K and S385T substitutions, typical for high-BLNAR isolates. Lack of product amplification using both abovementioned primer sets indicates low-BLNAR isolates. β-Lactamase production was detected by a chromogenic assay using nitrocefin as the substrate (Becton Dickinson, Sparks, MD).

In this study, results of the PCR screening along with the nitrocefin test allowed the isolates to be classified as: gBLNAR (genetically β-lactamase-negative, ampicillin-resistant; N526K and S385T substitutions or N526K alone detected with no β-lactamase production); gBLPACR (genetically β-lactamase-positive, amoxicillin/clavulanic acid-resistant; N526K and S385T substitutions or N526K alone detected with β-lactamase production); and gBLPAR (genetically β-lactamase-positive, ampicillin-resistant; wild-type PBP3 strain with no N526K and no S385T substitutions but with β-lactamase production preserved). Ampicillin-susceptible isolates (gBLNAS; genetically β-lactamase-negative, ampicillin-susceptible) were recognised if neither N526K/S385T substitutions nor β-lactamase were found.

2.3. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MIC) of ampicillin, amoxicillin, amoxicillin/clavulanic acid (AMC), cefuroxime, ceftriaxone and cefaclor were assessed by the broth microdilution (BMD) method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [22]. The disk diffusion method with ampicillin (2 μg), AMC (2/1 μg) and benzylpenicillin (1 U) was used to evaluate growth inhibition zones. The results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2016 guidelines [23]. *Haemophilus influenzae* ATCC 49766 (BLNAS), ATCC 49247 (BLNAR) and NCTC 8468 (BLNAS) were used for quality control as recommended by the CLSI and EUCAST.

The Nordic Committee on Antimicrobial Susceptibility Testing (NordicAST) guidelines were used to assess β-lactam resistance mechanisms in all tested isolates [24]. An inhibition zone of ≥12 mm in initial screening with the benzylpenicillin (1 U) disk indicated susceptibility. Subsequent testing for the presence of β-lactamases was carried out as a second step of the algorithm for isolates showing inhibition zones of <12 mm. A negative result of the nitrocefin assay equated to BLNAR identification, whilst the opposite result necessitated testing the isolate with a cefaclor disc (30 μg). A breakpoint of 23 mm in the growth inhibition zone was established to distinguish BLPAR from BLPACR strains.

2.4. DNA sequencing

The *ftsI* sequencing was performed for all gBLNAR and gBLPACR isolates based on the screening PCR results. A 705-bp fragment of the *ftsI* gene was amplified using the following primers with adaptors for sequencing: 5′-GTTTCCCAGTCACGACGTTGTAGTTAATGCGTAACCGTGCAATTAC-3′ and 5′-TTGTGAGCGGATAACAATTTCACCACTAATGCATAACGAGGATC-3′. The product was detected by standard gel electrophoresis and was then sequenced using a second set of primers (5′-GTTTTCCCAGTCACGACGTTGTA-3′ and 5′-TTGTGAGCGGATAACAATTTC-3′) [25]. The *ftsI* sequences were analysed using Lasergene 7 software (DNASTAR, Madison, WI) and were compared with the *ftsI* sequence of the *H. influenzae*

Table 1
Susceptibility data of different groups of *Haemophilus influenzae* isolates assessed by the broth microdilution method.

	gBLNAS (n = 76)	gBLNAR (n = 20)	gBLPAR (n = 21)	All (N = 117)
Ampicillin				
$MIC_{50/90}$ (mg/L)	0.25/0.5	2.0/2.0	>16/>16	0.5/>16
MIC range (mg/L)	0.06–1.0	0.5–4.0	16 to >16	0.06 to >16
% S/R[a]	100/0	35/65	0/100	70.9/29.1
Amoxicillin				
$MIC_{50/90}$ (mg/L)	0.25/0.5	2.0/4.0	>32/>32	0.5/>32
MIC range (mg/L)	0.06–2.0	0.5–4.0	>32 to >32	0.06 to >32
% S/R[a]	100/0	80/20	0/100	78.6/21.4
Amoxicillin/clavulanic acid				
$MIC_{50/90}$ (mg/L)	0.25/1.0	2.0/2.0	1.0/1.5	0.5/2.0
MIC range (mg/L)	0.12–2.0	0.25–4.0	0.25–2.0	0.12–4.0
% S/R[a]	100/0	90.0/10.0	100/0	98.3/1.7
Cefuroxime (intravenous)				
$MIC_{50/90}$ (mg/L)	0.5/1.0	2.0/2.0	0.5/2.0	1.0/2.0
MIC range (mg/L)	0.125–4.0	0.5–4.0	0.125–2.0	0.125–4.0
% S/I/R[a]	96.1/2.6/1.3	25.0/65.0/10.0	90.5/9.5/0	82.9/14.5/2.6
Ceftriaxone				
$MIC_{50/90}$ (mg/L)	≤0.0037/0.0075	0.015/0.03	0.0037/0.0075	0.0037/0.015
MIC range (mg/L)	≤0.0037–0.12	0.0037–0.03	≤0.0037–0.0075	≤0.0037–0.12
% S/R[a]	100/0	100/0	100/0	100/0
Cefaclor				
$MIC_{50/90}$ (mg/L)	2.0/4.0	8.0/8.0	8.0/16	2.0/8.0
MIC range (mg/L)	0.25–8.0	4.0–16.0	2.0–16.0	0.25–16
% S/R[a]	No EUCAST breakpoints			

gBLNAS, genetically β-lactamase-negative, ampicillin-susceptible; gBLNAR, genetically β-lactamase-negative, ampicillin-resistant; gBLPAR, genetically β-lactamase-positive, ampicillin-resistant; MIC, minimum inhibitory concentration; $MIC_{50/90}$, MIC for 50% and 90% of the isolates, respectively; S, susceptible; I, intermediate-resistant; R, resistant; EUCAST, European Committee on Antimicrobial Susceptibility Testing.
[a] Results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2016 guidelines [23].

Rd KW20 strain to detect nucleotide substitutions. Subsequent classification into different molecular groups was carried out as previously proposed [10,26].

3. Results

Of the 117 study isolates, 76 (65.0%) presented neither β-lactamase production nor PBP3 alterations and were recognised as gBLNAS, whereas 41 strains (35.0%) carried β-lactam resistance mechanism(s). β-Lactamase production together with lack of PBP3 alterations (gBLPAR) was detected in 21 isolates (17.9%). No amplification product was found in the PCR reaction using Hasegawa primers (gBLNAR) for 20 isolates (17.1%). No gBLPACR isolates were detected during the study.

Of the 117 isolates, 83 (70.9%) were susceptible to ampicillin, 92 (78.6%) to amoxicillin and 115 (98.3%) to AMC. Among 34 ampicillin-resistant isolates according to the BMD method results, 21 (61.8%) were gBLPAR and 13 (38.2%) were gBLNAR. Detailed characteristics of the isolates, along with in vitro susceptibility, MIC_{50}/MIC_{90} values and MIC range, are presented in Table 1.

In the gBLNAR group, five strains (25.0%) were isolated from patients with invasive infections. Four of these strains remained susceptible to ampicillin and one strain (from blood) was resistant (MIC = 2 mg/L). The remaining 15 gBLNAR strains (75.0%) were isolated from the LRT. Among them, 80.0% (12/15) were resistant to ampicillin (MIC >1 mg/L).

Among the 117 isolates, discrepancies in the susceptibility category for ampicillin were observed for 15 isolates (12.8%) and for AMC for 11 isolates (9.4%), depending on the method used (disk diffusion versus BMD). The results of susceptibility testing for ampicillin and AMC, along with the laboratory method used, are summarised in Table 2.

3.1. Evaluation of NordicAST algorithm and EUCAST screening disk test

The benzylpenicillin disc (1 U) discriminated BLNAS from BLNAR isolates in all cases, which was further confirmed for gBLNAR by *ftsI* sequencing. According to the algorithm, 76 strains (65.0%) did not carry any mechanism(s) of resistance to β-lactams (BLNAS), 20 strains were recognised as BLNAR, whilst the remaining 21 isolates demonstrated β-lactamase production. Following application of the cefaclor disk, 1 of 21 β-lactamase-producing strains was recognised as BLPACR based on an inhibition zone of <23 mm. Of note, the screening PCR as well as molecular sequencing did not confirm typical PBP alteration(s) in this strain; thus, it was finally recognised as gBLPAR.

3.2. Results of ftsI sequencing

Among the 117 study strains, 20 gBLNAR isolates (17.1%), identified based on the screening PCR, were subjected to partial *ftsI* sequencing. According to the proposed molecular classification, 19 isolates (95.0%) with altered PBP3 (gBLNAR) belonged to group II [10,26], representing four subgroups: IIa (n = 7; 35.0%); IIb (n = 8; 40.0%); IIc (n = 1; 5.0%); and IId (n = 3; 15.0%). The remaining single isolate belonged to the miscellaneous group of amino acid substitutions. In general, the most common amino acid

Table 2
Comparison of the susceptibility to ampicillin and amoxicillin/clavulanic acid (AMC) according to laboratory method used: disk diffusion (DD) versus broth microdilution (BMD).

Antibiotic	Genotype (n)	Method	Susceptible [n (%)]	Resistant [n (%)]
Ampicillin	gBLNAS (76)	DD	64 (84.2)	12 (15.8)
		BMD	76 (100)	0
	gBLPAR (21)	DD	0	21 (100)
		BMD	0	21 (100)
	gBLNAR (20)	DD	4 (20.0)	16 (80.0)
		BMD	7 (35.0)	13 (65.0)
	All (117)	DD	68 (58.1)	49 (41.9)
		BMD	83 (70.9)	34 (29.1)
AMC	gBLNAS (76)	DD	76 (100)	0
		BMD	76 (100)	0
	gBLPAR (21)	DD	19 (90.5)	2 (9.5)
		BMD	21 (100)	0
	gBLNAR (20)	DD	9 (45.0)	11 (55.0)
		BMD	18 (90.0)	2 (10.0)
	All (117)	DD	104 (88.9)	13 (11.1)

substitutions were N526K (20/20; 100%), D350N (16/20; 80.0%), and A502V, M377I and G490E (each 9/20; 45.0%). The *ftsI* gene substitutions detected in Polish strains are shown in Table 3.

Among all tested *H. influenzae* isolates, only two invasive isolates belonged to serotype b (Hib), including one gBLNAR of the most represented group IIb and one BLNAS. The remaining isolates were non-typeable *H. influenzae*.

3.3. Associations between PBP3 alterations and antimicrobial susceptibility

Among the 20 gBLNAR strains, 13 isolates (65.0%) were resistant in vitro to ampicillin (MIC >1 mg/L), of which 3 (23.1%) were classified in subgroup IIa (D350, G490, N526 and A530), 7 (53.8%) were in subgroup IIb (D350, M377, G490, A502 and N526), 1 (7.7%) was in subgroup IIc (A502 and N526) and 2 (15.4%) were in subgroup IId (I449 and N526). Among seven isolates susceptible in vitro to ampicillin, four belonged to subgroup IIa, whilst a single isolate represented the remaining categories (IIb, IId and the miscellaneous category).

4. Discussion

Among two the most common mechanisms of resistance to ampicillin in *H. influenzae*, β-lactamase production is usually easy to detect in the laboratory setting. Strains characterised by this mechanism demonstrate resistance when tested by ampicillin disk, their MICs are high above the resistance breakpoint and they are positive in the nitrocefin test [27]. BLNAR isolates with high MICs for ampicillin (e.g. 8–16 mg/L), described by some authors as high-BLNAR strains, are also easily identified by susceptibility testing [28]. However, in European countries, most BLNAR strains display low MICs for ampicillin, preserving susceptibility to ampicillin/AMC and belonging to the so-called low-BLNAR category [24]. The clinical significance of the distinction between BLNAS and low-BLNAR isolates remains unclear. Therapeutic choices rely on MIC values and, in most cases, low-BLNAR strains remain susceptible in vitro to aminopenicillins. However, increased vigilance should be paid to this category of strains since, due to lower susceptibility to β-lactams, a risk of clinical failure should be taken into consideration.

Several methods have been proposed to discriminate the resistance mechanisms of *H. influenzae* to β-lactams, including molecular identification of responsible genes. Despite this, classical phenotypic methods have an established position. NordicAST has proposed an easy-to-use algorithm for the detection of resistance to β-lactam antibiotics based on the EUCAST disk diffusion breakpoints [29]. In this study, we aimed to use this algorithm together with other methods to recognise mechanisms of resistance to β-lactams expressed by Polish *H. influenzae* isolates.

To the best of our knowledge, this is the first detailed study in Poland regarding mechanisms of resistance to β-lactams in *H. influenzae*, along with molecular aspects of such resistance. PCR screening and *ftsI* sequencing are not routinely used in Polish laboratories. Identification of resistance to β-lactams and its mechanisms is limited to the disk diffusion or antibiotic gradient test method and the nitrocefin test. Moreover, data regarding Polish *H. influenzae* strains are very scarce [12,30]. In the international study by Fluit et al., the resistance profile of Polish *H. influenzae* isolates was characterised by a higher proportion of BLNAR strains than in most other European countries [12]. Interestingly, a striking increase in BLNAR isolates was observed in Poland between the two collection periods (1997–1998 and 2002–2003) [12]. However, these results have to be interpreted with great caution due to insufficient representation, disparities in the quantity of strains collected in different countries (e.g. Poland was represented by 50 and 35 isolates in each of the periods studied) as well as fluctuations of the number of collected strains over time. The last study of the resistance patterns of selected respiratory tract pathogens in Poland was based on 344 *H. influenzae* collected between 2002 and 2004 [30]. This study revealed that 9% of *H. influenzae* isolates were β-lactamase-positive, almost 13% were identified as low-BLNAR, and only one isolate carried concomitantly two mechanisms of resistance and was recognised as BLPACR [30]. Comparing these last results with the present study, we have noticed an increase in the percentage of β-lactamase-positive isolates as well as of low-BLNAR.

In the current study, the ampicillin resistance rate was slightly higher than in a comparable Bulgarian analysis by Setchanova et al. (29.1% vs. 22%) [31]. Most Polish gBLNAR strains originated from the LRT but the percentages of gBLNAR among LRT and invasive isolates were similar (16.9% vs. 17.9%). In general, Polish *H. influenzae* strains exhibit the same mechanisms of resistance that appear in other European countries; however, certain infrequent mutation patterns have not been found in Polish BLNAR strains so far. All but one studied BLNAR isolate represented group II according to Dabernat's molecular classification [10]. Similarly, in a German analysis of ampicillin resistance mechanisms among invasive *H. influenzae* isolated between 2009 and 2012, most strains belonged to the same group II category. However, two isolates carried a mutation pattern fitting group III, with some additional mutations, and they have been attributed to group III-like [9,32]. In Portuguese and French analyses, such isolates have been also sporadically detected [33,34]. According to Skaare et al., clusters of group III and group III-like isolates with high-level resistance to third-generation cephalosporins were recently detected in Norway [35]. In all of the abovementioned studies, the vast majority of isolates belonged to group IIb, similarly to the current results. Also, observations of the Polish National Reference Center for Susceptibility Testing (NRCST; http://www.korld.edu.pl), based on isolates sent by hospital laboratories for confirmation of the BLNAR resistance mechanism,

Table 3
Amino acid substitutions in the *ftsI* gene of gBLNAR isolates ($n = 20$) according to molecular classification.[a]

Group	*n*	Amino acid substitutions for:											
		K344	D350	T352	K355	L356	M377	I449	G490	A502	V511	N526	A530
IIa	7		N						E			K	S
IIb	7		N				I			V		K	
	1		N				I		E	V		K	
IIc	1									V		K	
IId	3							V				K	
M[b]	1	R	N	G	T	V	I		E		A	K	

gBLNAR, genetically β-lactamase-negative, ampicillin-resistant.
[a] Isolates with mutations in the *ftsI* gene were classified into four groups: II (a, b, c and d) according to Dabernat et al. [10].
[b] One isolate with additional mutations in the *ftsI* gene.

revealed that from 78 *H. influenzae* isolates obtained in 2012, all 58 isolates confirmed as gBLNAR belonged to group II (IIa, $n = 7$; IIb, $n = 43$; IIc, $n = 2$; and IId, $n = 6$) (unpublished data). This is in line with a Swedish study by Resman et al. [16] and a Spanish paper by Puig et al. [36]. One of the isolates in the current study displayed an MIC of 4 mg/L for ampicillin with no mutation pattern representative for the high-BLNAR category. In this case the same substitutions were detected as in the gBLNAR isolates susceptible to ampicillin. This finding would suggest the presence of other, less common and/or unknown, mechanism(s) of resistance, which should be further explored.

All of the isolates included in this study were found to be susceptible to ceftriaxone, similarly to all Polish *H. influenzae* analysed up to now (unpublished data of the NRCST and the NRCBM). However, all gBLNAR strains demonstrated approximately two-fold higher MIC_{50} and MIC_{90} values for all antibiotics tested, including third-generation cephalosporins, compared with gBLNAS isolates. Similar results were obtained in a recent Swiss study [37]. Therefore, even if resistance to extended-spectrum cephalosporins is rare outside of Japan and South Korea [11,38], this phenomenon requires constant surveillance. It is especially important also in Europe since such isolates have been reported in Norway [35].

This study revealed noteworthy differences in the categorisation of strain susceptibility depending on the laboratory technique applied. The most striking differences were present within the gBLNAR category, which clearly indicates that the BMD and disk diffusion method are not fully interchangeable (Table 2). This problem was previously indicated by other investigators [39]. This is why it should be considered whether the increase of β-lactam resistance could be altered by the diagnostic approach used in a particular study. In most papers the disk diffusion method was preferred as the method of choice to study resistance to β-lactams [39,40]. According to our experience, the algorithm proposed by NordicAST with the benzylpenicillin disk (1 U), as also indicated by EUCAST, appears to be a good method representing a preliminary stage of β-lactam resistance identification. According to the algorithm, based on the cefaclor (30 μg) inhibition zone, the β-lactamase-producing isolates are subsequently classified as BLPAR or BLPACR. However, misclassification may occur; according to the algorithm, one isolate in the current study was classified as BLPACR while the PCR screening as well as sequencing did not confirm the typical amino acid substitution(s). Thus, in our opinion, the algorithm can be applied in routine laboratory work, whereas sequencing of the *ftsI* gene may be useful in molecular epidemiology studies.

Resistance to antibiotics constantly evolves and it is therefore necessary to carefully monitor the epidemiological situation. In the case of *H. influenzae* isolates it is especially important for β-lactams, used as first-line treatment, resistance to which requires continuous surveillance, effective detection methods and a rational policy of antibiotic usage.

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Competing interests

None declared.

Ethical approval

Not required.

Data availability

The data set is available from the corresponding author on reasonable request.

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