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Letter to the Editor

Emergence of cefotaxime-resistant *Haemophilus influenza* in Tunisia

Sir,

Resistance of *Haemophilus influenzae* to commonly used antibiotics is thought to be driven mainly by selective pressure brought about by antibiotic use. Resistance to β-lactams can be mediated by two well-known mechanisms. The most common mechanism involves the production of a β-lactamase enzyme, usually TEM-1-type, or more rarely ROB-1-type. Alterations in penicillin-binding protein 3 (PBP3) caused by mutations in the *ftsI* gene have also been reported in different *H. influenzae* strains [1]. According to the presence of specific amino acid substitutions in PBP3 encoded by the *ftsI* gene, these strains have been classified into three major groups (I–III). Isolates belonging to group III are normally associated with high resistance levels to ampicillin. Third-generation cephalosporins remain among the most active antibiotics [1,2]. However, frequent use of oral cephalosporins for the treatment of *H. influenzae* respiratory infections has led to the emergence of resistance to extended-spectrum cephalosporins [1,3].

Here we report the first strains of *H. influenzae* resistant to cefotaxime isolated in Tunisia and analyse their characteristics.

A total of 660 non-duplicate clinical *H. influenzae* strains were recovered in Sfax University Hospital (Sfax, Tunisia) between 2013 and 2017. Antimicrobial susceptibility testing was performed by the disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. β-Lactamase production was determined by the Cefinase^{TM} chromogenic test (bioMérieux). Minimum inhibitory concentrations (MICs) of cefotaxime were determined using Etest strips (AB BIODISK) for ampicillin-resistant strains. Among the 660 strains, 267 (40.5%) were resistant to ampicillin. Six strains were resistant to cefotaxime (MIC > 0.125 μg/mL). For these strains, MICs of ampicillin, amoxicillin, amoxicillin/clavulanic acid (AMC), cefixime, ceftriaxone and ceftaroline were evaluated by Etest and were interpreted according to EUCAST clinical breakpoints. The quality control strain used was *H. influenzae* ATCC 49766. Capsular serotyping was performed by *bexA* PCR [3]. Presence of the β-lactamase-encoding genes bla_{TEM-1} and bla_{ROB-1} was investigated by PCR amplification [2]. Alterations in PBP3 were investigated by sequencing the region of the *ftsI* gene encoding the transpeptidase domain of PBP3 for cefotaxime-resistant strains [2]. According to PBP3 substitution patterns and β-lactamase determination, strains were categorised into groups and resistance genotypes: β-lactamase-negative, ampicillin-resistant (BLNAR), i.e. strains that have mutations in the *ftsI* gene but did not produce a β-lactamase enzyme; and β-lactamase-positive, AMC-resistant (BLPACR), i.e. strains that have both β-lactamase production and amino acid substitutions in PBP3 [2,3].

The six cefotaxime-resistant strains lacked polysaccharide capsule genes and were categorised as non-typeable. These strains caused respiratory infections in patients with recurrent bronchopneumonia and repeated antibiotic consumption (AMC and oral cephalosporins) as reported in other studies [3,4]. Only one strain produced a β-lactamase (TEM-1). The six cefotaxime-resistant strains were also resistant to ampicillin, amoxicillin, AMC and cefixime. The MICs for cefotaxime were more than two times higher compared with the MICs for ceftriaxone (Table 1). Cefotaxime-resistant strains have been reported from Japan and Korea as well as in Europe [1,3,4]. According to data from the French national reference centre for *H. influenzae*, in France 11.5% of strains were resistant to cefotaxime (MICs of 0.25–1 μg/mL) in 2016 [5]. However, this resistance was increasing from 2011 (0.2%) to 2014 (2.5%) and was only observed in non-invasive isolates. Cefotaxime-resistant *H. influenzae* strains remain generally susceptible to ceftriaxone. The superiority of the intrinsic activity of ceftriaxone compared with cefotaxime has also been documented in other studies with, on average, two dilution values of difference in the MICs of the two antibiotics [4,5]. This explains the interest in using ceftriaxone for *H. influenzae* infections caused by cefotaxime-resistant strains.

The six cefotaxime-resistant strains presented mutations in the *ftsI* gene that have described in some previous studies [2–4]. Two strains were classified as group II, three strains belonged to group III-like and one strain belonged to group III (Table 1). In the literature, decreased susceptibility to extended-spectrum cephalosporins is generally associated with high-level PBP3-mediated resistance (high-rPBP3) group III, defined by the second-step substitution around the SSN motif S385T in addition to a first-step substitution around the KTG motif (R517H or N526K) [3,4]. The third-step substitution L389F is associated with further increased levels of resistance [3,4]. Among the two strains possessing the third-step substitution L389F in the current study, one was resistant to ceftriaxone. In Norway, isolates possessing the substitution L389F were generally more resistant to cephalosporins than isolates with high-rPBP3 and most of them were resistant to ceftriaxone [4]. In addition to groups III and III-like, group II was also implicated in resistance to cefotaxime in BLNAR strains isolated in Norway with clonal dissemination [4]. In Spain, cefotaxime-resistant strains belonged to groups IIa, IIb, IIc and III-like [3].

This is the first study performed in Tunisia regarding cefotaxime resistance in *H. influenzae*, an emerging phenomenon reported since 2013. Continuous surveillance is necessary to define trends in the antimicrobial resistance of *H. influenzae* in order to detect strains with high-level resistance to cephalosporins. Rational use of cephalosporins for the treatment of *H. influenzae* infections

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Table 1
Characteristics of cefotaxime-resistant *Haemophilus influenzae* strains in Tunisia.

Patient/year of isolation	Age (years)	Source	Clinical data	MIC (μg/mL)[a]							PCR for TEM-1	Co-resistance	Resistance genotyped	Amino acid substitutions in PBP3									Group
				AMP	AMX	AMC	CFM	CTX	CRO	CPT				Asp 350	Ser 357	Met 377	Ser 385	Leu 389	Ala 502	Arg 517	Asn 526	Thr 532	Ser 357	Val 547	
1/2013	69	Tracheal aspirate	Pneumonia	3	4	4	0.25	0.5	0.047	0.032	–	–	BLNAR	Asn		Ile			Val		Lys			Ile	IIb
2/2014	55	Tracheal aspirate	Pneumonia	1.5	3	4	2	0.5	0.064	0.094	–	–	BLNAR	Asn	Asn	Ile	Thr			His		Ser			III-like
3/2014	16	Sputum	AECB	1.5	4	4	0.25	0.5	0.047	0.064	–	SXT	BLNAR	Asn							Lys				IIa
4/2016	12	Sputum	Pneumonia	2	3	3	3	1.5	0.25	0.19	–	SXT	BLNAR	Asn	Asn	Ile	Thr	Phe		His		Ser			III-like
5/2016	31	Sputum	AECB	2	4	3	0.25	1	0.125	0.094	–	SXT	BLNAR	Asn	Asn	Ile	Thr	Phe	Thr		Lys				III
6/2017	8	Sputum	Pleuropneumonia	8	16	4	0.75	0.5	0.047	0.032	+	–	BLPACR	Asn	Asn	Ile	Thr			His		Ser			III-like

MIC, minimum inhibitory concentration; AMP, ampicillin; AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; CFM, cefixime; CTX, cefotaxime; CRO, ceftriaxone; CPT, ceftaroline; PBP, penicillin-binding protein; BLNAR, β-lactamase-negative, ampicillin-resistant; AECB, acute exacerbation of chronic bronchitis; SXT, trimethoprim/sulfamethoxazole; BLPACR, β-lactamase-positive, AMC-resistant.
[a] Interpreted according to EUCAST clinical breakpoints.

remains the best way to control the spread of these resistant strains.

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Competing interests

None declared.

Ethical approval

Not required.

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