Analysis of *ftsI* gene mutation in *Haemophilus influenzae* resistant to ceftriaxone: a single-center study, Seoul, Korea

Sanghoon Lee, Euntaek Lee, Gahee Kim, Jung Hwa Kim, Mina Kim, Jina Lee*
Department of Pediatrics, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea

INTRODUCTION

Haemophilus influenzae (*H. influenzae*) is a common causative organism of respiratory tract infection in young children.

Develops resistance to β-lactams by either producing β-lactamase or modifying the structure of penicillin-binding protein 3 (PBP3) by *ftsI* gene mutation.

Resistant to ceftriaxone has been continuously reported in our hospital since 2017.

The purpose was to identify the genotypic characteristics of ceftriaxone-resistant strains and to investigate the MIC differences accordingly.

METHODS

Inclusion criteria:

H. influenzae isolated from patients who visited Asan Children's Hospital from March 2017 to April 2019 and reported being resistant to ceftriaxone by disk diffusion method

Exclusion criteria:

Failed to a subculture or negative PCR for *ftsI* gene

Clinical information:

Age, sex, specimen collection date, source, underlying disease, antibiogram

Sequencing the *ftsI* gene and obtaining amino acid sequences:

Subculture and RNA extraction

Amplification of *ftsI* gene by PCR and sequencing

Comparing the sequences with index strain (Rd KW20) and checking the amino acids substitutions

Grouping by amino acid substitution patterns:

Classified according to Hasegawa et al., 2006

Obtaining ceftriaxone MIC by E-test

Identify the β-lactamase producers: by cefinase test and PCR for bla_{TEM} and bla_{ROB-1}

Breakpoints of *H. influenzae* for ceftriaxone (EUCAST 2022):

Susceptible range of breakpoint: ≤0.125 mg/L

PK-PD breakpoint: ≤1 mg/L

CONCLUSION

In South Korea, strains in group IV and IX has been increasing by the year and showed relatively high ceftriaxone MIC.

The strains with S385T showed significantly higher ceftriaxone MIC than the wild type.

Nevertheless, the clinical concerns about treatment failure are low since the MIC did not reach the therapeutic concentration of ceftriaxone (PK-PD breakpoint), ≤1 mg/L.

RESULTS

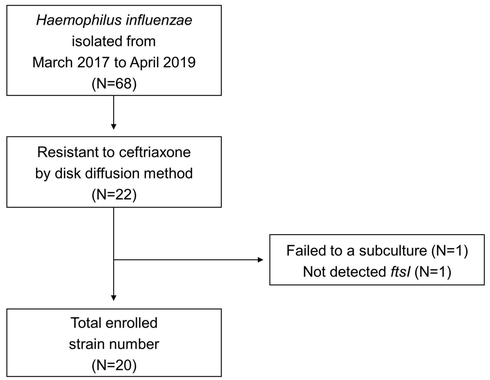

Figure 1. A flowchart showing the inclusion process.

Table 2. The annual detection number of each group according to amino acid substitution and β-lactamase producers

Group	Year (n, %)		
	2017	2018	2019
IV	1 (10)	2 (28.6)	0
V	3 (30)	0	0
VI	2 (20)	0	0
IX	4 (40)	5 (71.4)	3 (100)
β-lactamase producers	8 (80)	2 (28.6)	1 (33.3)

Table 2.

In 2017, all four groups were observed, whereas, in 2019, only group IX was observed.

The disappearance of the ceftriaxone-sensitive strains and the following S385T strains' predominance suggests that the trend of the ceftriaxone resistance mechanism is changing.

The number and proportion of β-lactamase producers decreased by the year.

All the β-lactamase producers were bla_{TEM}.(+), bla_{ROB-1} (-).

Table 1. Amino acid substitution patterns and corresponding groups, and the number of patients.

Group	n	Amino acid substitutions					Number of strains susceptible to antibiotics*				Minimum inhibitory concentration of ceftriaxone (mg/L) by E-test		
		M377	S385	L389	R517	N526	AMP	AMC	CXM	CRO	Median	Average	range
IV	3	I	T	F	H	-	0	0	0	0	0.250	0.397	0.190-0.750
V	3	-	-	-	-	K	0	3**	0	0	0.012	0.048	0.008-0.125
VI	2	-	T	-	-	K	0	1**	0	0	0.158	0.158	0.125-0.190
IX	12	I	T	F	-	K	0	0	0	0	0.190	0.210	0.190-0.250

*by disk diffusion method, **The four susceptible to amoxicillin/clavulanic acid also showed susceptibility to ceftriaxone by E-test.
Abbreviation: AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime; CRO, ceftriaxone; M, methionine; S, serine; L, leucine; R, arginine; N, aspartate; I, isoleucine; T, threonine; F, phenylalanine; H, histidine; K, lysine.

Table 1.

All strains could be classified into four groups (out of groups I to IX by Hasegawa et al.).

Most strains were basically resistant to the other three antibiotics.

Three in group V and one in group VI were susceptible to amoxicillin/clavulanic acid. The ceftriaxone MICs of those four were 0.125 mg/L or lower, which can be classified as ceftriaxone-susceptible.

The strains with S385T were observed in seventeen (85%), and all were included in groups IV, VI, and IX.

Either R517H or N526K was observed in all strains, but no one had both.

None of the strains reached the MIC of 1 mg/L, known as PK-PD breakpoints of ceftriaxone.

Figure 2.

(A) The median MIC was the same for three years. However, the minimum of the range increased since 2018.

(B) Group V showed the lowest median MIC, and group IV showed the highest.

(C) The strains with S385T showed significantly higher ceftriaxone MIC than wild type.

(D) The MIC of strains with R517H tended to be higher than those with N526K, but it was insignificant.

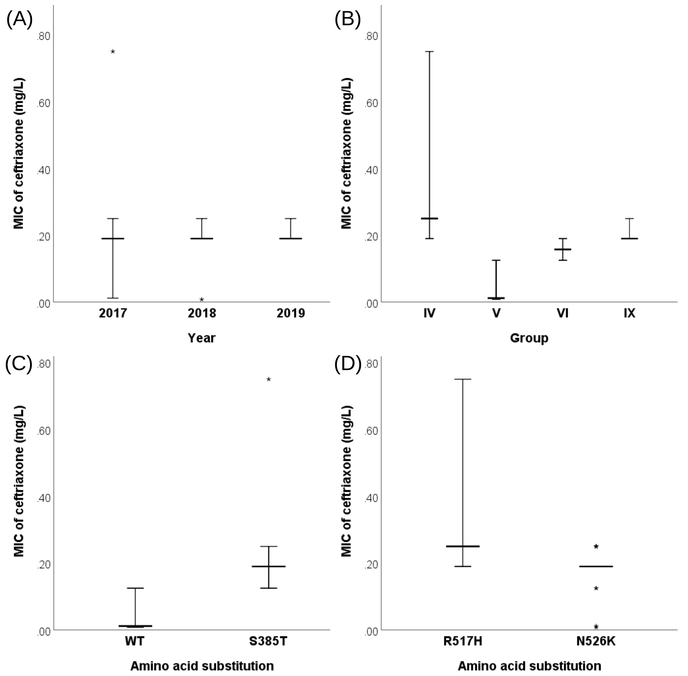

Figure 2. MIC of ceftriaxone by year (A), group according to amino acid substitution patterns (B), presence of S385T substitution (C), and presence of either R517H or N526K (D).
Long transverse bars indicate the median values and vertical bars indicate the range of MIC.