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ORIGINAL ARTICLE

Antibiotic Resistance of *Hemophilus influenzae* Isolated from Children in Southwest China

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SUMMARY

Background: ***Hemophilus influenzae*** **(*Hi*) is one of the major pediatric bacterial pneumonia pathogens that heavily threatens children's lives and global health. With widespread usage as first-line treatment, the prevalence of β-lactam-resistant strains is increasing sharply. In order to treat *Hi* more effectively, a systematic study on the antibiotic resistance profiles, β-lactamase-negative ampicillin-resistant (BLNAR) strains isolation rate, and potential BLNAR resistance mechanism in our region is needed.**

Methods: **This study analyzed antimicrobial susceptibility of *Hi*, and clinical data of *Hi*-infected patients retrospectively. BLNAR and β-lactamase-positive ampicillin-clavulanate resistant strains (BLPACR) were confirmed by the Kirby–Bauer method and β-lactamase test. *ftsI* gene in BLNAR was sequenced to find out whether resistance was induced by penicillin-binding protein mutation. Ampicillin susceptibility test with or without efflux pump inhibitors were done to assess efflux pump contribution in BLNAR. RT-PCR was performed to evaluate the efflux pump genes' transcription levels.**

Results: **A total of 2,561 *Hi* strains were isolated in our hospital from January 2016 to December 2019. Male to female ratio was 1.52:1. Median age was 10 months. Infant (< 3 years old) infection accounted for 83.72%. *Hi* resistance rates to sulfamethoxazole-trimethoprim, ampicillin, cefathiamidine, cefaclor, cefuroxime, cephalothin, amoxicillin-clavulanate, tetracycline, chloramphenicol, ofloxacin, cefotaxime, and rifampin were 84.28%, 78.01%, 49.80%, 41.98%, 36.58%, 33.64%, 4.55%, 4.1%, 3.37%, 1.77%, 0.99%, and 0.12%, respectively, while 1.33% were BLNAR. BLNARs were classified into four groups by mutation patterns in *ftsI* gene and most strains were divided to Group III/III-like. *EmrB*, *ydeA* and *norM* transcription levels in some ampicillin-resistant strains were higher than their sensitive counterparts.**

Conclusions: **Ampicillin is not sufficiently effective as a first-line *Hi* infection treatment. However, ampicillin-clavulanate and cefotaxime may be a better choice. Efflux pumps, *emrB*, *ydeA* and *norM* play roles in the high resistance to ampicillin.**

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Supplementary Data

hmrM primer design

Template: NC_000907.1 (*Hemophilus influenzae* Rd KW20, complete genome) Range1678071 - 1679465 (*hmrM* gene). Designed using SnapGene Viewer (version 3.1, Dotmatics, San Diego, California, USA) and validated by NCBI Primer BLAST and performing qPCR.
Forward primer: 5'-CGTGTAAATAATCGCGTGCT-3'. Reverse primer: 5'-GCGAGTGAGTTCCACTGATA-3'.
Product length: 284 bp.
Thermocycling conditions: 94°C for 10 seconds, 60°C for 30 seconds.

Table S1. BLNAR classification and MICs before and after EPIs.

Group	No.	Amino Acid Substitution Sites																						Ampicillin MIC (μg/mL)		
		Lys 344	Asp 350	Thr 352	Lys 355	Leu 356	Ser 357	Met 377	Ser 385	Leu 389	Val 461	Gly 490	Ala 502	Val 511	Arg 517	Ile 519	Asn 526	Ala 530	Thr 532	Val 547	Tyr 557	Val 562	Val 461	Before EPI	CCCP	PaβN
Sensitive strain																								1	0.5	0.5
II	ATCC49247																Lys			Ile			Ser	4	4	4
	13											Glu	Val				Lys			Ile			Ser	4	4	2
	17												Thr				Lys			Ile			Ser	8	4	4
	21		Asn									Glu					Lys	Ser						4	2	2
	23	Arg	Asn	Gly	Thr	Val						Glu	Val				Lys			Ile			Ser	4	2	2
	28		Asn										Thr				Lys							4	4	4
	29		Asn									Glu					Lys	Ser						16	2	1
III	1		Asn				Asn		Thr								Lys		Ser	Ile			Ser	4	2	2
	3		Asn				Asn	Ile	Thr	Phe		Glu	Val				Lys			Ile			Ser	16	16	16
	5		Asn				Asn	Ile	Thr	Phe							Lys						Ser	8	8	8
	6		Asn				Asn	Ile	Thr	Phe							Lys						Ser	32	8	4
	7		Asn				Asn		Thr								Lys		Ser	Ile			Ser	4	4	4
	10		Asn				Asn		Thr								Lys			Ile		Leu	Ser	16	8	4
	12		Asn				Asn	Ile	Thr	Phe							Lys			Ile		Leu	Ser	8	8	8
	14		Asn				Asn	Ile	Thr	Phe							Lys			Ile		Leu	Ser	8	8	8
	15		Asn				Asn	Ile	Thr	Phe							Lys			Ile		Leu	Ser	8	8	4
	20		Asn				Asn	Ile	Thr	Phe							Lys			Ile		Leu	Ser	8	8	8
	24		Asn				Asn	Ile	Thr	Phe							Lys			Ile		Leu	Ser	8	8	8
	31		Asn				Asn	Ile	Thr	Phe	Ile			Ala		Leu	Lys			Ile			Ser	8	4	4
	34		Asn				Asn	Ile	Thr	Phe	Ile			Ala		Leu	Lys			Ile				16	8	4
III-like	4		Asn				Asn	Ile	Thr	Phe					His				Ser	Ile	His		Ser	16	16	16
	18		Asn				Asn	Ile	Thr	Phe					His				Ser	Ile	His		Ser	8	2	4
	19		Asn				Asn	Ile	Thr	Phe					His				Ser	Ile	His		Ser	64	64	32
	22		Asn				Asn	Ile	Thr	Phe					His				Ser	Ile	His		Ser	8	4	4
	25		Asn				Asn	Ile	Thr	Phe					His				Ser	Ile	His		Ser	8	8	4
	27		Asn				Asn	Ile	Thr	Phe					His				Ser	Ile	His		Ser	64	64	32
	32		Asn				Asn	Ile	Thr	Phe					His				Ser	Ile	His		Ser	32	32	16
	33		Asn				Asn	Ile	Thr	Phe					His				Ser	Ile	His		Ser	16	16	16
Others	2		Asn				Asn										His					Leu		4	4	4
	8		Asn				Asn										His					Leu		4	4	4
	9		Asn				Asn										His					Leu		16	8	8
	11		Asn				Asn										His					Leu		8	4	1
	16		Asn				Asn										His					Leu		4	2	4
	26		Asn				Asn										His					Leu		4	4	4
	30		Asn				Asn										His					Leu	Ser	16	16	8

BLNAR were classified into four groups by mutation patterns in *ftsI* gene. Group III accounted for the largest (13/34, 38.23%), Group III-like for 23.53% (8/34), Group II for 17.65% (6/34) and other substitutions for 20.59% (7/34). CCCP: carbonyl cyanide m-chlorophenylhydrazone, PaβN: phenylalanine-arginine β-naphthylamide, EPI: Efflux Pump Inhibitor, Lys: Lysine, Arg: Arginine, Asp: Aspartic acid, Asn: Asparagine, Thr: Threonine, Gly: Glycine, Leu: Leucine, Ile: Isoleucine, Val: Valine, Ser: Serine, Met: Methionine, Phe: Phenylalanine, Glu: Glutamic acid, Ala: Alanine, His: Histidine, Tyr: Tyrosine.