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Clonal Spread of Non-Typable *Haemophilus influenzae* in Long-Term Ventilator Units: High Prevalence of bla_{ROB-1} Without Ceftriaxone Resistance

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Background

Over the past two years, the repeated isolation of *Haemophilus influenzae* from respiratory and blood specimens in two long-term ventilator care units, respiratory care ward A and B (RCW-A and RCW-B) of our hospital, prompted an investigation into potential clonal dissemination. This study aimed to assess the genetic relatedness and antimicrobial resistance profiles of the isolates.

Materials and methods

Specimens were collected between January and May 2025. A total of 49 *Haemophilus influenzae* isolates: 24 clinical strains obtained from 22 patients (RCW-A: 3 patients; RCW-B: 2; other wards: 17) and 25 strains recovered through active respiratory screening of ventilated patients (RCW-A: 21/25 [84%]; RCW-B: 4/25 [16%]). Species identification was performed using the Bruker MALDI-TOF MS system. Genotyping was conducted by pulsed-field gel electrophoresis (PFGE). For each patient, demographic and clinical data were collected, including age, sex, hospital ward at the time of culture, and the source specimen of each isolate.

Results

Analyzed 49 Haemophilus influenzae isolates. Genotyping via pulsed-field gel electrophoresis (PFGE) revealed 15 distinct pulsotypes. Notably, 23 of 24 isolates from RCW-A clustered into two dominant pulsotypes (Groups J and O), and 7 of 7 isolates from RCW-B clustered into Groups L and M, supporting localized transmission. In contrast, 18 isolates from other wards exhibited greater genetic diversity (10 pulsotypes). All isolates tested negative for *bexA*, classifying them as non-typable *Haemophilus influenzae* (NTHi). Resistance gene analysis showed a high prevalence of bla_{TEM-1} (44/49; 89.8%) of 49 *Haemophilus influenzae* isolates and a markedly higher rate of bla_{ROB-1} carriage in RCW isolates (13/31; 41.9%) compared to non-RCW units (2/18; 11.1%). Importantly, all isolates remained susceptible to ceftriaxone (MIC range <0.016–0.38 μg/mL).

Conclusion

These findings suggest ongoing clonal transmission of non-typable *Haemophilus influenzae* (NTHi) in our long-term ventilator units, coupled with increased carriage of bla_{ROB-1}. Although ceftriaxone resistance was not observed, continued molecular surveillance and targeted infection control interventions are warranted to mitigate further spread.

Table 1. The antimicrobial susceptibilities of 49 *Haemophilus influenzae* isolates.

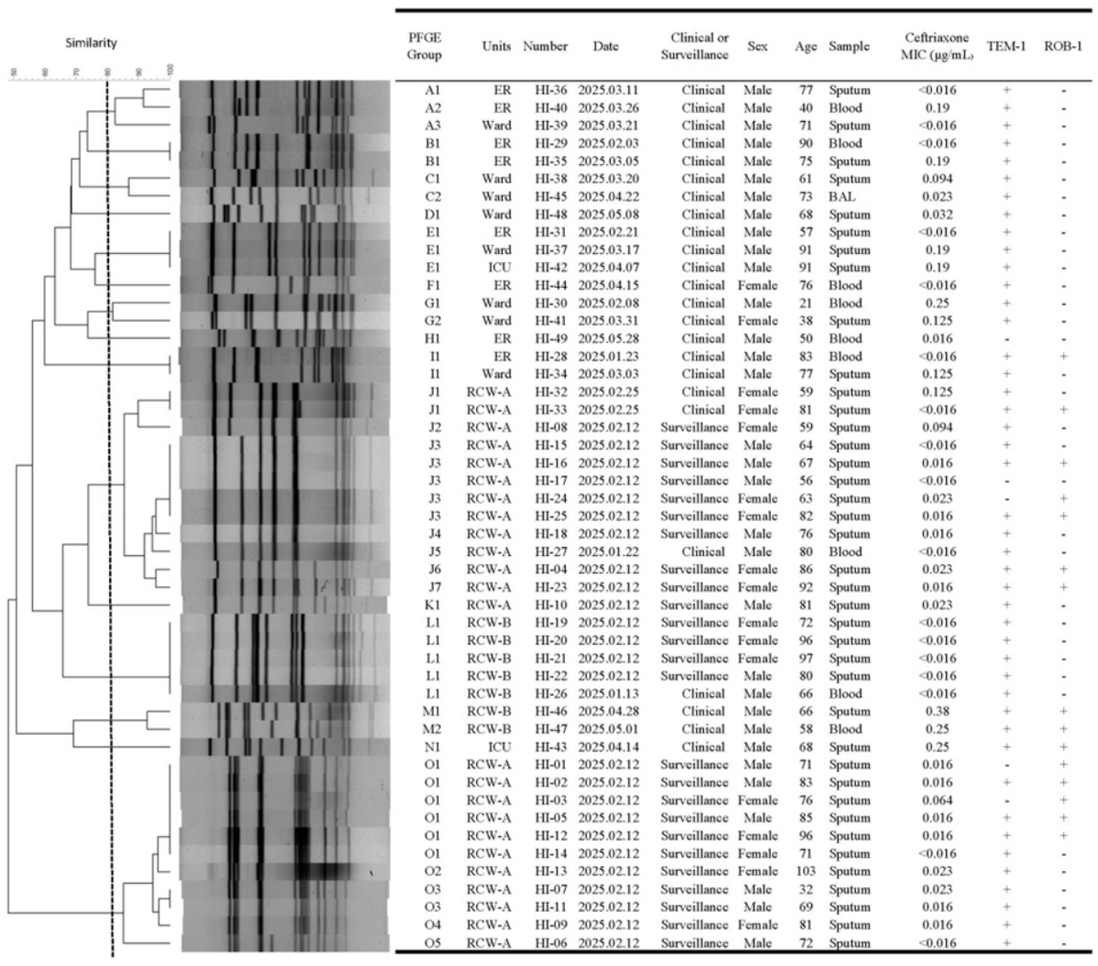
	[a]RCW (n=31)			non-RCW (n=18)		
Antibiotic	Resistance rate, n (%)			Resistance rate, n (%)		
	R	I	S	R	I	S
Amikacin	14(45.2%)	0(0%)	17(54.8%)	18(100%)	0(0%)	0(0%)
Cefuroxime	9(29.0%)	1(3.2%)	21(67.8%)	14(77.8%)	1(5.6%)	3(16.6%)
Ceftriaxone	2(6.5%)	0(0%)	29(93.5%)	0(0%)	0(0%)	18(100%)
Chloramphenicol	4(12.9%)	0(0%)	27(87.1%)	2(11.1%)	0(0%)	16(88.9%)
Rifampin	3(9.7%)	0(0%)	28(90.3%)	1(5.6%)	0(0%)	17(94.4%)
[b]SXT	28(90.3%)	0(0%)	3(9.7%)	11(61.1%)	0(0%)	7(38.9%)

Interpretation criteria were based on the Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility testing standards.**Abbreviations:** R, resistant; I, intermediate, S, susceptible ;
[a]RCW:Respiratory care ward ;[b]SXT: Sulfamethoxazole / Trimethoprim.

Table 2. Antimicrobial resistance gene distribution of 49 *Haemophilus influenzae* isolates.

	Resistance gene		
	bexA	[b]bla_{TEM-1}	bla_{ROB-1} **carriage**
[a]RCW (n=31)	0(0%)	27(87.1%)	13(41.9%)
non-RCW (n=18)	0(0%)	17(94.4%)	2(11.1%)

Abbreviations: [a]RCW: Respiratory care ward ; [b]*bla*: beta-lactamase .



PFGE Group	Units	Number	Date	Clinical or Surveillance	Sex	Age	Sample	Ceftriaxone MIC (μg/mL)	TEM-1	ROB-1
A1	ER	HI-36	2025.03.11	Clinical	Male	77	Sputum	<0.016	+	-
A2	ER	HI-40	2025.03.26	Clinical	Male	40	Blood	0.19	+	-
A3	Ward	HI-39	2025.03.21	Clinical	Male	71	Sputum	<0.016	+	-
B1	ER	HI-29	2025.02.03	Clinical	Male	90	Blood	<0.016	+	-
B1	ER	HI-35	2025.03.05	Clinical	Male	75	Sputum	0.19	+	-
C1	Ward	HI-38	2025.03.20	Clinical	Male	61	Sputum	0.094	+	-
C2	Ward	HI-45	2025.04.22	Clinical	Male	73	BAL	0.023	+	-
D1	Ward	HI-48	2025.05.08	Clinical	Male	68	Sputum	0.032	+	-
E1	ER	HI-31	2025.02.21	Clinical	Male	57	Sputum	<0.016	+	-
E1	Ward	HI-37	2025.03.17	Clinical	Male	91	Sputum	0.19	+	-
E1	ICU	HI-42	2025.04.07	Clinical	Male	91	Sputum	0.19	+	-
F1	ER	HI-44	2025.04.15	Clinical	Female	76	Blood	<0.016	+	-
G1	Ward	HI-30	2025.02.08	Clinical	Male	21	Blood	0.25	+	-
G2	Ward	HI-41	2025.03.31	Clinical	Female	38	Sputum	0.125	+	-
H1	ER	HI-49	2025.05.28	Clinical	Male	50	Blood	0.016	-	-
I1	ER	HI-28	2025.01.23	Clinical	Male	83	Blood	<0.016	+	+
I1	Ward	HI-34	2025.03.03	Clinical	Male	77	Sputum	0.125	+	-
J1	RCW-A	HI-32	2025.02.25	Clinical	Female	59	Sputum	0.125	+	-
J1	RCW-A	HI-33	2025.02.25	Clinical	Female	81	Sputum	<0.016	+	+
J2	RCW-A	HI-08	2025.02.12	Surveillance	Female	59	Sputum	0.094	+	-
J3	RCW-A	HI-15	2025.02.12	Surveillance	Male	64	Sputum	<0.016	+	-
J3	RCW-A	HI-16	2025.02.12	Surveillance	Male	67	Sputum	0.016	+	+
J3	RCW-A	HI-17	2025.02.12	Surveillance	Male	56	Sputum	<0.016	-	-
J3	RCW-A	HI-24	2025.02.12	Surveillance	Female	63	Sputum	0.023	-	+
J3	RCW-A	HI-25	2025.02.12	Surveillance	Female	82	Sputum	0.016	+	+
J4	RCW-A	HI-18	2025.02.12	Surveillance	Male	76	Sputum	0.016	+	-
J5	RCW-A	HI-27	2025.01.22	Clinical	Male	80	Blood	<0.016	+	-
J6	RCW-A	HI-04	2025.02.12	Surveillance	Female	86	Sputum	0.023	+	+
J7	RCW-A	HI-23	2025.02.12	Surveillance	Female	92	Sputum	0.016	+	+
K1	RCW-A	HI-10	2025.02.12	Surveillance	Male	81	Sputum	0.023	+	-
L1	RCW-B	HI-19	2025.02.12	Surveillance	Female	72	Sputum	<0.016	+	-
L1	RCW-B	HI-20	2025.02.12	Surveillance	Female	96	Sputum	<0.016	+	-
L1	RCW-B	HI-21	2025.02.12	Surveillance	Female	97	Sputum	<0.016	+	-
L1	RCW-B	HI-22	2025.02.12	Surveillance	Male	80	Sputum	<0.016	+	-
L1	RCW-B	HI-26	2025.01.13	Clinical	Male	66	Blood	<0.016	+	-
M1	RCW-B	HI-46	2025.04.28	Clinical	Male	66	Sputum	0.38	+	+
M2	RCW-B	HI-47	2025.05.01	Clinical	Male	58	Blood	0.25	+	+
N1	ICU	HI-43	2025.04.14	Clinical	Male	68	Sputum	0.25	+	+
O1	RCW-A	HI-01	2025.02.12	Surveillance	Male	71	Sputum	0.016	-	+
O1	RCW-A	HI-02	2025.02.12	Surveillance	Male	83	Sputum	0.016	+	+
O1	RCW-A	HI-03	2025.02.12	Surveillance	Female	76	Sputum	0.064	-	+
O1	RCW-A	HI-05	2025.02.12	Surveillance	Male	85	Sputum	0.016	+	+
O1	RCW-A	HI-12	2025.02.12	Surveillance	Female	96	Sputum	0.016	+	+
O1	RCW-A	HI-14	2025.02.12	Surveillance	Female	71	Sputum	<0.016	+	-
O2	RCW-A	HI-13	2025.02.12	Surveillance	Female	103	Sputum	0.023	+	-
O3	RCW-A	HI-07	2025.02.12	Surveillance	Male	32	Sputum	0.023	+	-
O3	RCW-A	HI-11	2025.02.12	Surveillance	Male	69	Sputum	0.016	+	-
O4	RCW-A	HI-09	2025.02.12	Surveillance	Female	81	Sputum	0.016	+	-
O5	RCW-A	HI-06	2025.02.12	Surveillance	Male	72	Sputum	<0.016	+	-

Figure 1. The dendrogram of 49 *Haemophilus influenzae was* constructed based on PFGE patterns and prevalence of. bla_{TEM-1} and bla_{ROB-1} carriage gene.

Reference

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