

MATERIALS AND METHODS 2

From 1999 to 2008, we obtained nasopharyngeal swab specimens from children between 5 months and 3 years of age attending a same day care center in Kanazawa city

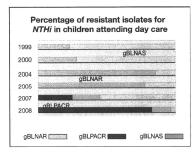
in 1999 (n=34)
2000 (n=42)
2004 (n=32)
2005 (n=36)
2007 (n=41)
2008 (n=31)

Nasopharyngeal carriage of respiratory pathogens in children attending day care

Year	swabs	No. (%) of SP	No. (%) of NTHi
1999 (Feb)	34	33 (97.1)	26 (76.5)
2000 (Feb)	42	28 (66.7)	45 (92.9)
2004 (Aug)	32	22 (68.8)	14 (43.8)
2005 (Feb)	36	32 (88.9)	27 (75.0)
2007 (May)	41	37 (90.2)	40 (97.6)
2008 (May)	31	23 (74.2)	29 (93.5)

Percentage of resistant isolates for NTHi in children attending day care

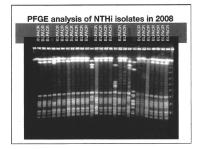
No. (%)	Total No of NTHi	gBLNAS	gBLNAR	gBLPACR		
1999	26	20 (76.9)	6 (23.1)	0		
2000	39	28 (71.8)	11 (28.2)	0		
2004	14	2(14.3)	12 (85.7)	0		
2005	27	6 (16.7)	21 (58.3)	0		
2007	40	22 (53.7)	8 (19.5)	10 (24.4)		
2008	29	1 (3.2)	4 (12.9)	24 (77.4)		



Pulse-Field Gel Electrophoresis (PFGE)

- In conventional gels, the current is applied in a single direction (from top to bottom). But in PFGE, the direction of the current is applied in two directions.
- PFGE allows investigators to separate much larger pieces of DNA than conventional agarose gel electrophoresis.





MIC distributions for eight antimivrobial agents against 24 isolates of gBLPACR (upper), 3 isolates of gBLNAR (middle) and 2 isolates of gBLNAS (lower) in 2008.

	PCR-based geostype	Se, of boleto with MOC control										MIC tracell			
		\$9,000	0.915	4-03	0.66	0335	6.35	65	1	2	*	۴	Range	59%	90%
COTR-PI	gHLPACE gHLNAR gHLNAS	1	1	1	24		1	Г				Г	0.06 0.03-0.15 5.0008-0.011	2.00 2.00 2.0005	9 0s. 9 25 0.003
AMPC	STERACE SEENAR SEENAS						1	;	1		1	23	48 0.54 0.35-0.3	8 1 9.25	2 05
MSA	gBLPACE gBLNAR gBLNAS					1	1	2	7	1		Г	8125-1 93-02 939-65	0.5 0.25	2 05
ABPC	gBLDACE gBLNAR gBLNAS					Г		2 2	П	280-0) 1 (+2)	Г		95-02 0.5	92 65 65	12 12 03

CONCLUSIONS

- Most of the children attending a day care had resistant strains of NTHi with PBP gene mutations in their nasopharynx.
- gBLPACR strains in day care children have been rapidly increased since 2007.
- PFGE analysis demonstrated that all gBLPACR strains were clonally identical.
- This represents the first report of the apparent clonal spread of gBLPACR strain of NTHi occurred in a certain condition.
- Clonal dissemination of gBLPACR observed in this study might be an alarm of increasing more serious bacterial infectious diseases in the future.