Emerging Beta-lactamase-negative Ampicillin Resistant *Haemophilus influenzae* in Japan and South Africa

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Introduction

Haemophilus influenzae (HINF) remains an important cause of lower respiratory tract infection worldwide, and resistance to ß-lactams is emerging in many countries. We examined the susceptibility of HINF to a broad range of antimicrobials as part of the SENTRY worldwide surveillance program in the Asia-Pacific region (17 hospitals in 8 countries).

Methods

Isolates

Isolates of HINF in the SENTRY surveillance program were collected over defined seasonal intervals between 1998 and 2002 from a range of sources, including respiratory secretions and blood. All strains were sent to a central reference laboratory (Women's and Children's Hospital, Adelaide, Australia) for testing

Susceptibility testing

All isolates were tested by broth microdilution using custom made panels (TREK™ Diagnostic Systems), according to NCCLS standards,[1] to a range of antimicrobials. Breakpoints for resistance were those recommended by the NCCLS[2] where available

Beta-lactamase Group

Isolates were classified according to β-lactamase production and ampicillin (AMP) susceptibility. β-lactamase-negative (BLN) ampicillin-resistant (BLNAR) PCR classification was as described by Ubukata.[3]

Results

- Over 100 isolates were available for testing from each country except mainland China (n=23) and the Philippines (n=3) where this organism was an infrequent clinical isolate
- The majority of isolates were from upper (82%) and lower (15%) respiratory tract
- β-lactamase production ranged from 6% of HINF from Japan to 68% in Taiwan (Table 1)
- BLN strains with AMP MIC of 2 mg/L (10.5%) and >2 mg/L (9.7%) accounted for over 20% of all isolates from Japan
- 76% of ampicillin-non-susceptible HINF in Japan were beta-lactamase-negative (Figures 1 and 2). These strains also gave significant rates of resistance to cefuroxime (14.1% non-susceptible) (Figure 3)
- Similar strains were detected in all other countries, except Taiwan, however none had AMP MIC >2 mg/L (Figure 2)
- Cephalosporin MIC distributions were significantly elevated for BLNAR strains (Figures 4-6). Comparative

Figure 1. Ampicillin Resistance Mechanism by Country

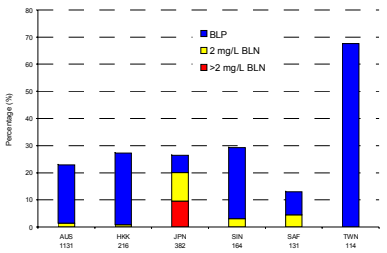
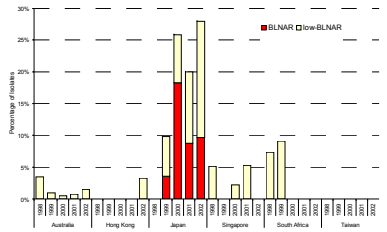


Figure 2. Incidence of BLNAR (1998-2002)



Results (Continued)

MIC_{50} and MIC_{90} values are shown in Table 2

- All isolates that had AMC MIC > 2 mg/L and BLN strains with AMP MIC >1 mg/L were classified by PCR (Table 3 and 4)
- Two amoxicillin/clavulanate-resistant (MIC ≥ 8 mg/L) strains had both TEM-1 β-lactamase and BLNAR amino acid substitutions. Also, 25 strains contained low-BLNAR mutations and TEM-1
- Resistance to tetracycline was low except in Taiwan (53%) and Hong Kong (15%); resistance to trimethoprim-sulfamethoxazole was common in most countries except Japan (6%)
- Resistance to chloramphenicol was high in Taiwan (49%), Hong Kong China (15%) and Singapore (9%)

Table 1. Resistance by Country

Country	N	β-lac	Amp >2	Amc >4	Cfu >8	Chl >4	Tet >4	SXT >0.5
Australia	1131	21.5	21.4	0.1	0.2	1.9	1.9	11.1
Hong Kong	216	26.4	26.4	0	0	14.9	15.3	25.9
Japan	381	6.3	16.0	8.1	13.6	2.0	2.9	6.0
Singapore	164	26.2	26.2	0	0	8.6	6.7	25.0
South Africa	131	8.4	8.4	0	0	1.5	1.5	32.8
Taiwan	114	67.5	67.5	0	0	48.6	52.6	58.8

Figure 4. Cefpodoxime MIC Distribution

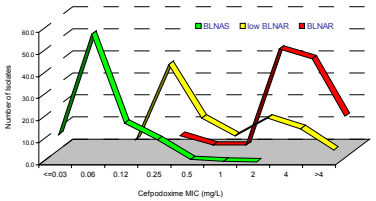


Figure 5. Cefepime MIC Distribution

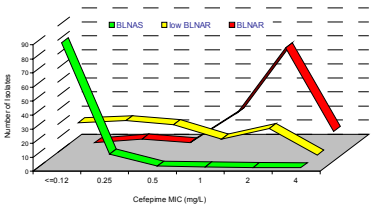
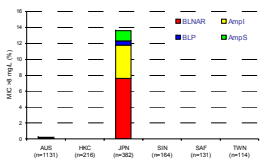


Figure 3 Ampicillin Phenotype

3a. Cefuroxime Resistance



3b. Amoxicillin/clavulanate Resistance

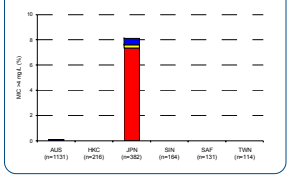


Figure 6. Cefuroxime MIC Distribution

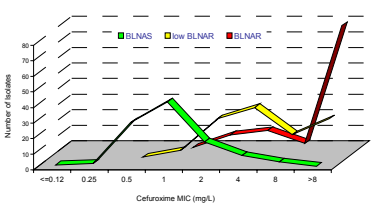


Table 2. Comparative MIC_{50} and MIC_{90}

Agent	BLNAS N	BLNAS MIC_{50}	BLNAS MIC_{90}	lowBLNAR N	lowBLNAR MIC_{50}	lowBLNAR MIC_{90}	BLNAR N	BLNAR MIC_{50}	BLNAR MIC_{90}	BLP N	BLP MIC_{50}	BLP MIC_{90}
Ampicillin	1601	≤0.5	1	69	2	2	37	4	8	456	>4	>16
Amoxi/clav	1601	0.5	≤2	69	2	4	37	8	>8	456	1	2
Cefuroxime	1602	1	4	69	4	>8	37	>8	>16	456	1	2
Cefepime	1602	≤0.12	0.25	69	0.5	2	37	2	2	456	≤0.12	0.12
Cefpodoxime	1352	0.06	0.25	62	0.5	4	23	4	>4	388	0.06	0.25
Ceftriaxone	1117	≤0.008	≤0.25	49	0.06	0.25	37	≤0.25	0.25	344	≤0.008	≤0.25

Table 3. Ampicillin vs Amoxicillin/clavulanate MICs

Ampicillin	Amoxicillin/clavulanate BLN	≤2	4	8	>8	Total
	≤1	1598	3			1601
	2	38	30	1		69
	≥4		9	22	6	37
	Total	1636	42	23	6	1707

Amoxicillin/clavulanate BLP	≤4	≥8	Total
≤1	0	0	0
2	1	0	1
≥4	452	3	455
Total	459	3	456

Table 4. Amoxicillin/clavulanate MIC vs PCR Group

AMC	N	BLNAS	low-BLNAR group I	low-BLNAR group II	BLNAR	BLNAR TEM-1*	low-BLNAR TEM-1
≤2	129	3	10	97	17		2
4	64		1	17	24		22
≥8	32			2	27	2	1
Total	225	3	11	116	68	2	25

* BLPACR

References

1 NCCLS. Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria That Grow Aerobically, 4th Ed. Approved Standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa

2 NCCLS. Performance standards for Antimicrobial Susceptibility Testing; 13th Informational Supplement. M100-S13. NCCLS 20012 Wayne, Pa

3 Ubukata, K et al. 2001. Antimicrob. Agents Chemother. **45:**1693-1699

Acknowledgments

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Conclusions

- **There is marked regional variation in the resistance patterns of HINF in the Asia-Pacific region**
- **Beta-lactamase negative-ampicillin-resistant *H. influenzae* are a significant problem in Japan. Low-BLNAR isolates are also found in several other countries in our region**
- **Elevated cephalosporin and/or amoxicillin/clavulanate MICs may indicate the presence of BLNAR**