

## The prevalence and antibiotic susceptibility of *Haemophilus influenzae* in the Emergency Intensive Care Unit of Kitasato University Hospital

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**Objective:** *Haemophilus influenzae*, 1 of 3 major pathogenic bacteria causing community-acquired pneumonia, is showing an increasing resistance to antibiotics, which is problematic in patients with various infections. The prevalence of drug-resistant *H. influenzae* was measured to evaluate its impact on patients admitted to the Emergency Intensive Care Unit (EICU).

**Methods:** Sputum samples were taken from patients with a suspected diagnosis of pneumonia admitted to the EICU of Kitasato University Hospital from April 2014 through December 2016. The microscopic examination of those samples included in this study showed: Geckler classification IV–V, phagocytosis, and  $\leq 10^6$  colonies when cultured. Of the 257 isolated clones, 17 were *H. influenzae* strains. It was the second most common bacterial species after methicillin-susceptible *Staphylococcus aureus*.

**Results:** The detection rate of drug-resistant *H. influenzae* strains was 75% (3/4 strains), 71.4% (5/7 strains), and 50% (2/4 strains) in 2014, 2015, and 2016, respectively. The resistance rate to sulbactam/ampicillin was 53.3% (8/15 strains), piperacillin was 13.3% (2/15 strains), and clarithromycin was 20.0% (3/15 strains).

**Conclusions:** The *H. influenzae* clones detected in the EICU showed high level of resistance to many of the common antimicrobials used for its treatment. Community-acquired pneumonia and early ventilator-associated pneumonia caused by antibiotic resistant bacteria is common. The presence of drug-resistant *H. influenzae* must be considered upon selecting antibiotics for patients with pneumonias in EICU settings.

**Key words:** *Haemophilus influenzae*, antibiotic susceptibility, Intensive Care Unit

### Introduction

Recently, the mortality by pneumonia has rapidly increased.<sup>1</sup> Since November 2010, nation-wide vaccination against *Streptococcus pneumoniae* and *Haemophilus influenzae* has been implemented. As a result, the incidences of invasive pneumococcal diseases (IPD) and invasive *H. influenzae* disease (IHD) have decreased.<sup>2</sup> However, in hospital settings, immediate intervention is required for patients with bacterial pneumonia, particularly for patients in intensive care

units, as there is concern that the prognosis will worsen if the initial antibiotic administered is ineffective. However there is no report for antimicrobial resistant *H. influenzae* in emergency intensive care settings. Therefore, we investigated the prevalence of bacteria in sputum samples obtained from patients with suspected pneumonias upon hospitalization at the Emergency Intensive Care Unit (EICU). Of these bacteria, we examined the prevalence and antibiotic susceptibility of *H. influenzae*, which is showing an increase in drug resistance<sup>3,4</sup> and is 1 of 3 major pathogenic bacteria causing community-acquired

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pneumonia. This research was conducted with the approval of the Kitasato University ethics committee (B17-023).

## Materials and Methods

Two hundred fifty-seven specimens of sputum obtained from pneumoniae patients, admitted to the EICU of Kitasato University Hospital from April 2014 through December 2016, were included in the study. The samples that showed Geckler classification IV to V on microscopic examination and phagocytosis, were included in this study. The first sputum specimen obtained from the patient was included in the study and all these were collected within 4 days of admission. Intubated patients submitted sputum specimens after intubation take down. All patients submitted sputum specimens before antibacterial treatment. A total of 138 cases were included in this study, and each case consisted of 1 patient where  $\geq 1$  bacterial species were detected. Of the 138 samples, we isolated 257 strains as general bacteria that showed phagocytosis on microscopic examination and  $\geq 10^6$  colonies<sup>5</sup> on isolation culture, making them eligible for investigation of pneumonia. Seventeen *H. influenzae* clones were identified from the 257 samples; however, 2 clones could not be recovered and were excluded from the study. For *H. influenzae* differentiation, XV Multidisc (Eiken) and horse blood were added to the blood agar medium used for identification of the pathologic bacteria.

The 15 strains of *H. influenzae* were classified into 5 groups according to ampicillin (ABPC) resistance,<sup>6</sup> as recommended by the Clinical and Laboratory Standards Institute (CLSI), as follows: (1)  $\beta$ -lactamase non-producing ABPC-sensitive strain (BLNAS), with a minimum inhibitory concentration (MIC) of  $\leq 1 \mu\text{g/ml}$  to

ABPC, as non- $\beta$ -lactamase-producing strain; (2)  $\beta$ -lactamase non-producing ABPC intermediately resistant strain (BLNAI), with an MIC of  $2 \mu\text{g/ml}$  to ABPC, as non- $\beta$ -lactamase-producing strain; (3)  $\beta$ -lactamase-negative ampicillin-resistant strain (BLNAR) with an MIC to ABPC of  $\geq 4 \mu\text{g/ml}$ , as non- $\beta$ -lactamase-producing strain; (4)  $\beta$ -lactamase-producing ABPC-resistant strain (BLPAR) with an MIC to sulbactam (SBT)/ABPC of  $\leq 4 \mu\text{g/ml}$ , as  $\beta$ -lactamase-producing strain; and (5)  $\beta$ -lactamase producing clavulanic acid/amoxicillin (CVA/AMPC)-resistant strain (BLPACR) with an MIC to SBT/ABPC of  $>4 \mu\text{g/ml}$ .  $\beta$ -lactamase-productivity was determined using BD BBL Cefinase Paper Discs (Becton-Dickinson Microbiology Systems) by the nitrocefin method. For antibiotic susceptibility of *H. influenzae*, MICs were determined for the following antibiotics: SBT/ABPC, piperacillin (PIPC); ceftriaxone (CTRX); levofloxacin (LVFX); ceftazidime (CAZ); clarithromycin (CAM); meropenem (MEPM). Thereafter, patient background and antibiotic susceptibility of the antimicrobials used for the initial treatment were examined in the patients with *H. influenzae* infection. MIC was measured using RAMH1 plate (Nissui Pharmaceutical, Tokyo), a fully automatic identification sensitivity measuring device based on the microdilution method.

## Results

### 1. The prevalence of general bacteria

The bacterial species that had more than 10 strains were detected were as follows: Methicillin-susceptible *Staphylococcus aureus* (*S. aureus*) (73 strains); *H. influenzae* (17 strains); *Streptococcus pneumoniae* (*S. pneumoniae*) (14 strains); *Streptococcus mitis* (13 strains);

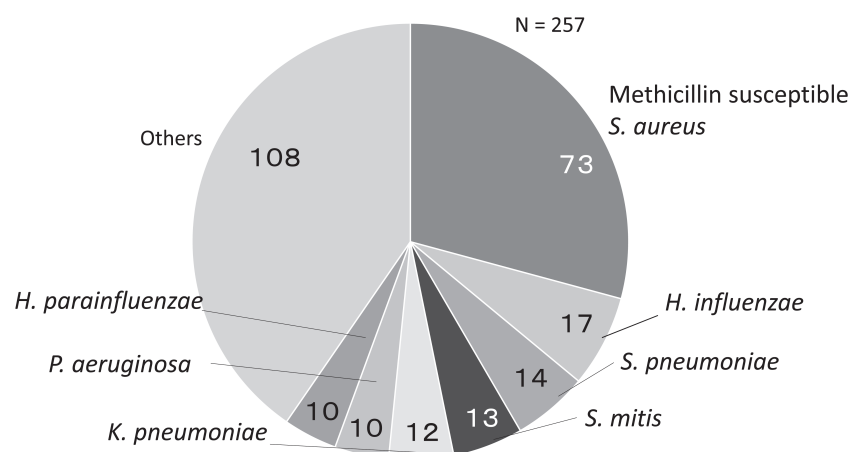


Figure 1. Bacterial strains in the EICU

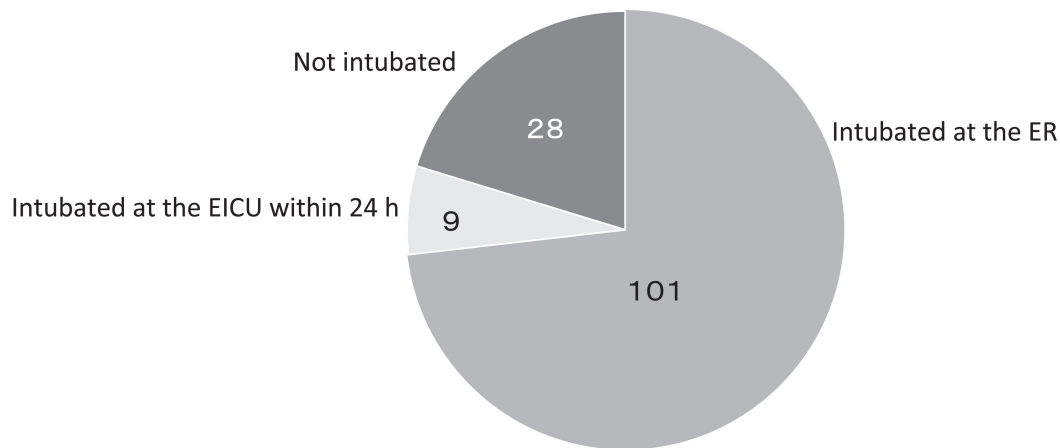
*Klebsiella pneumoniae* (12 strains): *Pseudomonas aeruginosa* (*P. aeruginosa*), and *H. parainfluenzae* (10 strains). *H. influenzae* was the second most prevalent bacteria in the isolated clones (Figure 1).

### 2. Airway management of the 138 patients

For 101 of 138 patients requiring airway management, endotracheal intubation was performed in the ER; for 9 patients, endotracheal intubation was performed within 24 hours after admission to the ER. Therefore, management with a mechanical ventilator was initiated early for 110 patients (Figure 2).

3. Background of the patients with *H. influenzae* infection  
The mean age of the patients was 47.3 years (15–74 years). The disease severity according to the mean

sequential organ failure assessment (SOFA) score was 4.86 (2 minimum; 9 maximum), and the mean Acute Physiology and Chronic Health Evaluation (APACHE II) score was 28.06 (11 minimum; 39 maximum). Therefore, the group consisted of patients with high severity. In many cases, low or no consciousness was observed and management with mechanical ventilation was required, while pneumonia itself was not the case of hospitalization for any of the cases. SBT/ABPC, tazobactam/piperacillin (TAZ/PIPC), CTRX, and CAZ were chosen for treatment. A single drug was used for all patients except for 1 patient who was treated with a combination of vancomycin with TAZ/PIPC. Three patients did not undergo any antimicrobial drug treatment (Table 1).



**Figure 2.** Airway management

**Table 1.** Number of strains and patient characteristics

Strains No.	Type	Antibiotics	Age (47.3) Sex	Diagnosis	SOFA (4.86)	APACHE II (28.06)	Place of Intubation
1	BLNAS	None	15 M	Intracranial Hemorrhage	3	11	ER
2	BLNAI	SBT/ABPC	70 M	Trauma	7	39	ER
3	BLNAR	TAZ/PIPC	74 M	Post CPA	4	29	ER
4	BLNAI	TAZ/PIPC	73 F	SAH	4	27	ER
5	BLNAI	None	52 F	Trauma	4	24	ER
6	BLNAI	CAZ	25 M	Intracranial Hemorrhage	7	26	ER
7	BLPACR	SBT/ABPC	66 F	Post CPA	3	35	ER
8	BLNAI	SBT/ABPC	40 F	Hypoxia	4	28	ER
9	BLNAS	TAZ/PIPC	47 M	Intracranial Hemorrhage	8	39	Not intubated
10	BLNAS	None	20 M	Drug addiction	4	20	Not intubated
11	BLPACR	CTRX	27 F	Drug addiction	2	15	Not intubated
12	BLNAS	SBT/ABPC	72 M	Post CPA	3	33	ER
13	BLNAR	VCM + TAZ/PIPC	25 M	Drug addiction	9	33	ER
14	BLNAS	SBT/ABPC	70 M	Post CPA	7	37	ER
15	BLNAR	SBT/ABPC	33 M	Post CPA	4	25	ER

4. The annual ABPC resistance prevalence of *H. influenzae*

In 2014, there were 4 strains: BLNAS (1), BLNAR (1), and BLNAI (2). In 2015, there were 7 strains: BLNAI (3), BLNAS (2), and BLPACR (2). In 2016, there were 4 strains: BLNAS (2) and BLNAR (2). No BLPAR strains were detected in any of the 3 years. The prevalence of ABPC-resistant bacteria was as high as 50% each year (Figure 3).

5. MIC distribution of various antibacterial drugs for *H. influenzae*

For BLNAS, 1 of 5 strains showed resistance to clarithromycin (CAM) at  $\geq 32 \mu\text{g/ml}$ . For BLNAR, 1 of

3 strains showed moderate resistance to CAM at  $16 \mu\text{g/ml}$ , and 1 strain showed resistance to MEPM at  $1 \mu\text{g/ml}$ . For BLNAI, of the 5 strains, 3 strains showed resistance to SBT/ABPC at  $\geq 4 \mu\text{g/ml}$ , and 1 strain was resistant to CAM at  $16 \mu\text{g/ml}$ . For BLPACR, 1 of 2 strains showed resistance to PIPC at  $\geq 4 \mu\text{g/ml}$ . The resistance ratio of the 15 strains of *H. influenzae* was 53.3% (8 strains), 13.3% (2 strains), and 20.0% (3 strains) for SBT/ABPC, PIPC, and CAM, respectively (Table 2).

Discussion

*H. influenzae* is known as a major pathogen causing infections, such as community-acquired pneumonia, acute

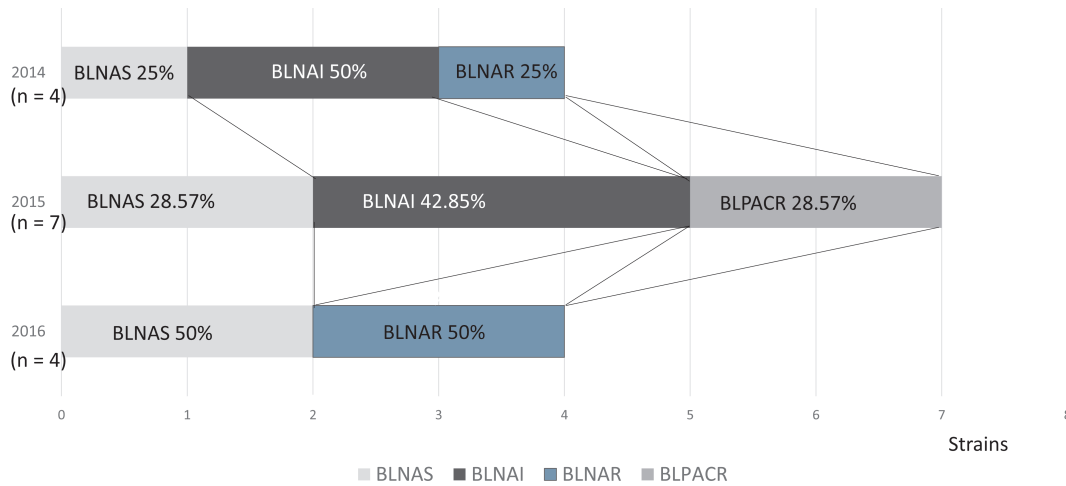


Figure 3. Change of resistance rates for *H. influenzae*

Table 2. Distribution of MICs of various antibacterial drugs in 5 groups of *H. influenzae*

Strains	Types	n	Antibacterial drugs																
			ABPC		SBT/ABPC		PIPC*		CTRX	LVFX		CAZ		CAM		MEPM			
			S	I	R	S	R	S	R	S	—	S	—	S	—	S	—		
BLNAS	5	5			5		5		5		5		4		1	5			
BLNAR	3			3		3		3		3		3		2	1	2	1**		
BLPAR	0																		
BLNAI	5		5		2	3	5	5		5		5		4	1	5			
BLPACR	2			2		2		2		2		2		2		2			
Total	15	5	5	5	7	8	13	2	15		15		15		12	2	1	14	1
Resistance rate (%)			66.7			53.3		13.3	0.0		0.0		0.0		22.2			6.7	

\*Reference determination value of MIC for tazobactam/piperacilin

\*\*MIC value:  $1 \mu\text{g/ml}$

middle otitis, and bacterial meningitis. Recently, the resistance to various  $\beta$ -lactams has made it difficult to treat these conditions making this a major problem. Since 1998, the incidence of BLNARs, as  $\beta$ -lactamase non-producing bacteria with ABPC resistance, has increased rapidly.<sup>7</sup> BLNARs are very common, and this has become a problem for respiratory infection, especially in pediatric patients.<sup>8</sup> In the present study, we examined the impact of such resistant bacteria in the EICU.

We found that the *H. influenzae* population in our study was comprised of both bacteria that are generally drug susceptible causing community pneumonia and drug-resistant bacteria that tend to cause nosocomial pneumonia. Contrary to our expectations, the majority of the strains showed resistance to one or more of the antibiotics tested, indicating the possibility of drug-resistant strains circulating in the community.

There may be two possibilities for this trend of resistance. First, drug-resistant *H. influenzae* may already be prevalent in the community. Second, because the patients admitted to the EICU are in critical or severe conditions, they may have already undergone previous antibiotic treatment, which leads to an increase in drug-resistant bacteria colonization in the patient. However, no association was found between intubation and severity of drug resistance for *H. influenzae*. Most of these patients met the criteria for pneumonia occurring after 48 hours following the use of a mechanical ventilator, defined as ventilator-associated pneumonia.

In addition, broad-spectrum penicillin and third-generation cephem antibiotics were mainly used, many as a monotherapy. By direct microscopic examination of the sputum, pneumonia was suspected in the 3 patients with no antibiotic use. However, they were not clinically diagnosed with pneumonia. Considering the background that SBT/ABPC and CTRX were used in most of the patients because of the severity of the cases within 4 days after admission, it appeared that the risk of colonization and infection of multidrug-resistant bacteria was low<sup>9</sup> (Table 3).

For pneumonia cases considered not to be of risk of resistant bacteria within 4 days of hospitalization, we used of the recommended antimicrobials, such as SBT/ABPC, CTRX, and LVFX, which are considered effective against community-acquired pneumonia as described in the guidelines of The Japan Respiratory Society.<sup>10</sup>

For PIPC, both strains of BLPACR showed resistance. In a report of BLNAR by Ubukata et al.,<sup>11</sup> the construction of penicillin binding protein 3 (PBP3) was changed by *fts I* gene mutations, resulting in a decrease of drug affinity, which led to reduction of the susceptibility to

$\beta$ -lactams other than ABPC. This phenomenon was thought to occur in BLPACR strains with similar characteristics and  $\beta$ -lactamase productivity. As mentioned above, it has recently been reported that resistance in *H. influenzae* tends to increase because of the changes in the construction of PBP3, resulting in resistance to  $\beta$ -lactams and  $\beta$ -lactamase production.<sup>12</sup> Moreover, most PBP3 mutations are caused by abuse of oral third-generation cephem antibiotics, which is considered specific to Japan.

For CAM, resistance was shown in 1 of 5 strains of BLNAS, 1 of 3 strains of BLNAR, and 1 of 5 strains of BLNAI. Unlike  $\beta$ -lactams, macrolide antibiotics are associated with the pump mechanism due to drug efflux proteins. In addition, a novel resistance mechanism due to ribosomal protein mutation has also recently been found.<sup>13</sup> Macrolide antibiotics have a high molecular weight and show poor outer membrane permeability of *H. influenzae*, a gram-negative bacillus. Therefore, it would appear that the antibacterial activity is lower in comparison with cephem antibiotics and quinolone antibacterial agents.

One of 2 strains of BLNAR and 1 of 15 (5.7%) strains of *H. influenzae* showed an MIC of 1  $\mu$ g/ml to MEPM. No factor that induces resistant bacteria was found in the background of this strain. BLNAR is resistant to  $\beta$ -lactams other than penicillin drugs due to its resistance mechanism. Furthermore, Sawada et al.<sup>14</sup> reported that its sensitivity was inferior to CTRX; and, Oikawa et al.<sup>15</sup>

**Table 3.** Risk factors for multidrug-resistant pathogens

MDR VAP
Prior intravenous antibiotic use $\leq$ 90 days
Septic shock at time of VAP
ARDS preceding VAP
$\geq$ 5 days of hospitalization prior to VAP
Acute renal replacement therapy prior to VAP
MDR HAP
Prior intravenous antibiotic use $\leq$ 90 days
MRSA VAP/HAP
Prior intravenous antibiotic use $\leq$ 90 days
Pseudomonas VAP/HAP
Prior intravenous antibiotic use $\leq$ 90 days

ARDS, acute respiratory distress syndrome; HAP, hospital-acquired pneumoniae; MDR, multidrug-resistant; MRSA, methicillin-resistant *S. aureus*; VAP, ventilator-associated pneumoniae



reported that regarding throat and nasal cavity cleaning fluid for pediatric outpatients in 2016, BLNAR showed an MIC of 1  $\mu$ g/ml to MEPM detected 5.3% (17/318), which was similar to our results.

In the present study, the SBT/ABPC resistance ratio of *H. influenzae* was as high as 53.3% (8/15 strains). Generally speaking, gram-negative bacteria are not considered because the identification of the bacterial species is difficult compared to that of gram-positive bacteria by smear examination of sputum. However, considering the high frequency of SBT/ABPC-resistant *H. influenzae*, when small gram-negative bacilli are suspected by direct smear examination of the sputum, it may be necessary to administer antimicrobials other than SBT/ABPC as an empirical therapy in a single agent. It is also considered that SBT/ABPC is inappropriate as the drug of choice for empirical therapy for patients admitted to the EICU under circumstances when a direct smear examination of sputum cannot be performed. For LVFX, only 1 of 10 *P. aeruginosa* strains was resistant, and all *H. influenzae* were sensitive in this study. Therefore, it was thought that the use of LVFX as an empirical therapy was appropriate in the presence of SBT/ABPC-resistant *H. influenzae*. On the other hand, because there are several reports<sup>16-18</sup> purporting that *H. influenzae* are resistant to quinolone antibacterial agents, it is necessary to pay careful attention to the future trends.

Delayed or inappropriate treatment of infection greatly affects the patients and can result in critical condition by sepsis caused by various pathogens such as gram-negative bacilli.<sup>19</sup> Therefore, carbapenems are often chosen in situations such as septic shock. However, the use of broad-spectrum antimicrobials such as carbapenems for all cases of infection may increase the risk of drug resistance in bacteria, including multidrug resistance in *P. aeruginosa*.<sup>20-22</sup> Although clinically uneventful at this stage, considering the actual situation under which susceptibility of *H. influenzae* to MEPM decreases, as stated above, greater considerations should be required before choosing carbapenems as an empirical therapy for pneumonia in patients admitted to the EICU. Therefore, physicians involved in emergency intensive care should determine the primary causative organism on the basis of the results of direct smear examination of sputum and choose the appropriate antimicrobials after having considered the presence of primary causative organisms of community-acquired pneumonia and of drug-resistant bacteria.

The results of this study show that *H. influenzae*, one of the representative pathogenic bacteria of community-acquired pneumonia, was often detected in the EICU and

that *H. influenzae* exhibits high levels of antimicrobial resistance. These findings must be carefully considered when antimicrobials are chosen for the initial treatment of patients with pneumonia admitted to the EICU.

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**Conflicts of Interest:** None

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