

Isolation and Antimicrobial Susceptibility Testing of *Haemophilus influenzae* in a Tertiary Care Hospital, a 6-Year Study (2016 to 2021)

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Objective: To determine the prevalence and antimicrobial susceptibility of *Haemophilus influenzae* at Taksin Hospital.

Materials and Methods: The susceptibilities of clinical isolates to six antimicrobial agents, ampicillin (AP), cefuroxime (CXM), cefotaxime (CTX), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT), and meropenem (MEM), between 2016 and 2021 were evaluated by the standard disk diffusion method.

Results: One hundred thirty-eight *H. influenzae* clinical isolates were found in different patients. There were 63.04% male patients. The ages of patients ranged from two months to 93 years old (mean±SD: 65.67±20.58). *H. influenzae* was mostly found in patients older than 50 (60.14%) years old. In children, *H. influenzae* was mostly found in patients younger than 1 to 10 (16.67%) years old. Unduplicated *H. influenzae* from different patients were isolated from the sputum (71.01%), blood (11.59%), pus from eyes (10.14%), and urethra (2.90%). *H. influenzae* demonstrated 100% susceptibility to CTX, CIP, and MEM, but less susceptibility to CXM (93.48%), AP (49.28%), and SXT (53.62%).

Conclusion: The present study results suggested the importance of monitoring the prevalence of *H. influenzae* at a hospital in Bangkok. The antibiogram of susceptibility helps provide guidelines for clinician to consider empirical treatment.

Keywords: *Haemophilus influenzae*; Drug resistance, Thailand

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Haemophilus influenzae is an important bacteria as it is responsible for causing various diseases in humans such as community-acquired pneumonia, meningitis, bacteremia, acute epiglottitis, otitis media, sinusitis, and conjunctivitis⁽¹⁻³⁾. In Europe, 3,882 cases of invasive *H. influenzae* disease were reported in 2018⁽⁴⁾, or 0.8 cases per 100,000

population, an increase from 0.6 per 100,000 in 2014⁽⁴⁾. The highest rate of infection was patients younger than one with 4 cases per 100,000 population, followed by patients aged 65 years and older with 2.4 cases per 100,000 population⁽⁴⁾. Community-acquired pneumonia is the most common disease associated with *H. influenzae*. Multiple drug resistance to *H. influenzae* has previously been reported^(3,5). Patterns of *H. influenzae* antimicrobial susceptibility may vary according to country, geography, patient age, and infection site.

The aim of the present study was to evaluate the prevalence and drug resistance of *H. influenzae* isolated in patients at Taksin Hospital, a public tertiary care hospital with 500 beds in central Bangkok operated by the Bangkok Metropolitan Administration. The present study determined antibiogram pattern profiles to provide guidance on the treatment of *H. influenzae* disease to clinicians.

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Materials and Methods

The present study was conducted after ethical approval was obtained from the Human Research Committee at Siam University, reference code SIAMPY-IRB 2020/011.

Bacterial isolates and identification procedure

One hundred and thirty-eight *H. influenzae* isolates were collected from different patients to prevent duplicates of the same antibiogram profile at Taksin Hospital between January 2016 and December 2021. *H. influenzae* was isolated from clinical specimens using standard microbiological methods. If the specimen was sputum and contained more than 25 polymorphonuclear cells and less than 25 squamous epithelial cells per low-power field (10×10 microscope magnification), it was accepted for culture. *H. influenzae* was identified on typical colony morphology and confirmed by X and V factors for growth⁽²⁾.

Antimicrobial susceptibility testing

All *H. influenzae* isolates were tested for antimicrobial susceptibility to 10 µg ampicillin, 30 µg cefuroxime, 30 µg cefotaxime, 5 µg ciprofloxacin, 1.25/23.75 µg trimethoprim-sulfamethoxazole, and 10 µg meropenem by the disk diffusion method as described by the Clinical Laboratory Standards Institute (CLSI)⁽⁶⁾. The bacterial inoculum was prepared using the *Haemophilus* colony suspension method in which colonies from an overnight culture, for 20 to 24 hours, on chocolate blood agar. The turbidity of inoculum was adjusted to the 0.5 McFarland standard. *Haemophilus* test medium (Oxoid, UK) was incubated at 35°C in 5% CO₂ for 16 to 18 hours. *H. influenzae* ATCC 49247 was used as quality control. The criteria for interpretation were susceptible, intermediate, and resistant according to CLSI recommendation⁽⁶⁾.

Data analysis

Data were entered and analyzed using IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA) for descriptive analyses. Categorical variables, such as age of patients and source of clinical specimens were expressed as frequency and percentages.

Results

In the present study, there were 39, 25, 27, 28, 16, and 3 isolates in 2016, 2017, 2018, 2019, 2020, and 2021, respectively. Eighty-seven patients (63.04%)

Table 1. Prevalence and source of specimens of *H. influenzae*

	No. of patients (n=138); n (%)
Age groups (years)	
<1 to 10	23 (16.67)
11 to 20	6 (4.35)
21 to 30	7 (5.07)
31 to 40	11 (7.97)
41 to 50	8 (5.80)
51 to 60	18 (13.04)
61 to 70	24 (17.39)
71 to 80	22 (15.94)
>80	19 (13.77)
Specimens	
Sputum	98 (71.01)
Blood	16 (11.59)
Pus from eyes	14 (10.14)
Urethral swab	4 (2.90)
Throat swab	2 (1.45)
Nasal swab	2 (1.45)
Bronchial wash	1 (0.72)
Vagina swab	1 (0.72)

were male and 51 (36.96%) were female. Hence, the male to female ratio was 1.71 to 1. The age of patients ranged from two months to 93 years old. The mean age and standard deviation were 65.67±20.58 years old. *H. influenzae* was mostly found in patients aged older than 50 (60.14%) years old (Table 1). In children, *H. influenzae* was mostly found in patients younger than 1 to 10 years (16.67%). The age group with the lowest number of patients was adults aged 21 to 50 (5.07% to 7.97%) years old. The sources of clinical specimens are shown in Table 1, and the three most common were sputum (71.01%), blood (11.59%), and pus from eyes (10.14%).

In microbiology laboratory reports, the amount of *H. influenzae* isolated on chocolate blood agar by the streaked plate technique, for various clinical specimens except blood culture, ranged from numerous growths, moderate growth, and a few colonies of *H. influenzae*. In the present study, the authors evaluated the quantity of *H. influenzae* grown from all clinical specimens, except blood (138–16=122). *H. influenzae* was found to have numerous growths on chocolate blood agar at 81.15% (99/122), moderate growth at 17.21% (21/122), and a few colonies at 1.64% (2/122).

Antimicrobial susceptibility testing

H. influenzae was susceptible to ampicillin (49.28%), trimethoprim-sulfamethoxazole (53.62%),

Table 2. Antimicrobial susceptibility of *H. influenzae* by the disk diffusion method

Antimicrobial agents	No. of isolates; n (%)		
	Susceptible	Intermediate	Resistant
Ampicillin	68 (49.28)	16 (11.59)	54 (39.13)
Cefuroxime	129 (93.48)	-	9 (6.52)
Cefotaxime	138 (100)	-	-
Ciprofloxacin	138 (100)	-	-
SXT	74 (53.62)	5 (3.62)	59 (42.76)
Meropenem	138 (100)	-	-

SXT=trimethoprim-sulfamethoxazole

and cefuroxime (93.48%). It also demonstrated 100% susceptible to cefotaxime, ciprofloxacin, and meropenem (Table 2).

Discussion

During the present study period, the number of *H. influenzae* isolates declined sharply due to the introduction of measures to prevent the spread of COVID-19 in Thailand in early 2020. The male to female ratio in the present study was 1.71 to 1, which was higher than a previous report in which the ratio ranged between 0.9 to 1 and 1.2 to 1.0 between 2012 and 2015 at Siriraj Hospital⁽⁵⁾. Clinical specimens were highest in the sputum at 71.01%, obtained from the lower respiratory tract. The present study investigation confirmed the result of other studies in which *H. influenzae* was mostly isolated from clinical specimens of the sputum, indicating that the respiratory tract is the most common site of infection caused by this organism⁽⁷⁾. *H. influenzae* transmission usually occurs via droplets. Neonates may become infected by swallowing amniotic fluid during delivery or through contacts with vaginal secretions containing living *H. influenzae*⁽⁸⁾. The authors found the prevalence of *H. influenzae* in the vagina and urethra to be 0.73% and 2.90%, respectively (Table 1). Without an effective and early treatment, *H. influenzae* may cause serious complications such as bacteremia or meningitis. Bacteremia may lead to surgical amputation of limbs while meningitis can lead to permanent hearing loss, neurological sequelae, permanent brain damage, and even death. Cases of invasive *H. influenzae* disease continue to occur sporadically. In the present study, there were 16 blood isolates (11.59%) from 16 patients. However, the authors did not find any *H. influenzae* isolates in the cerebrospinal fluid.

By using the disk diffusion method, the high percentage of ampicillin resistance in the present

study may be due to beta-lactamase production⁽⁵⁾. Globally, reports show a varied geographic prevalence of ampicillin resistance in *H. influenzae* from 10% to 58.10%^(5,7,9,10). Production of beta-lactamase by plasmids carrying beta-lactamase genes is the most common mechanism leading to beta-lactam resistance. Two types of beta-lactamase, which are TEM-1 and ROB-1 types, have been described in *H. influenzae*, but TEM-1 is predominant (93.7%)⁽⁷⁾. However, *H. influenzae* is 99%⁽⁵⁾ susceptible to amoxicillin/clavulanate because clavulanate is a potent beta-lactamase inhibitor.

In the present study, 42.76% of *H. influenzae* was resistant to trimethoprim-sulfamethoxazole (Table 2). This value is lower than a previous report from another hospital in Thailand⁽⁵⁾ that found 47% of *H. influenzae* to be resistant to trimethoprim-sulfamethoxazole. Interestingly, the authors found 6.52% of *H. influenzae* to be resistant to cefuroxime, which was higher than a previous report from another hospital in Thailand⁽⁵⁾ that noted 1% of *H. influenzae* as being resistant to cefuroxime. In a study from Taiwan, resistant percentage of *H. influenzae* to cefuroxime was 7.6%⁽³⁾.

Presently, there is a dearth of information regarding the resistance to cefotaxime and meropenem. Recently, one multicenter study in China revealed that 5.9% and 0.2% of *H. influenzae* isolates from children were resistant to cefotaxime and meropenem⁽¹⁰⁾. Meanwhile, a meta-analysis of database in Iran (Pubmed, Scopus, and web of Science) showed that *H. influenzae* was 22.3% and 23.2% non-susceptible to cefotaxime and ciprofloxacin, respectively⁽¹¹⁾. In general, *H. influenzae* carries plasmids encoding beta-lactamase enzymes. However, another beta-lactam resistance mechanism that has been described are the mutations of the *ftsI* gene in the *H. influenzae* chromosome^(3,12). The *ftsI* gene encodes penicillin-binding protein 3 (PBP3), leading to an altered PBP that has a low affinity for binding to ampicillin and cephalosporin. Between 2019 and 2020 in Tunisia, there were two reports of the emergence of *H. influenzae* isolates that were cefotaxime-resistant^(13,14). However, until now, there have not been any genetic studies on the drug resistant genes of *H. influenzae* isolated in patients at Taksin Hospital. It will be useful to assay these drug resistant genes in other *H. influenzae* isolates from patients at Taksin Hospital to elucidate the precise molecular characterization and clonal spread.

Data from the National Antimicrobial Resistant Surveillance Center, Thailand for *H. influenzae* (1,032

isolates) from all specimens in 83 hospitals, between January and December 2020 show percentages of susceptibility to ampicillin at 61.6%, amoxicillin-clavulanate at 92.8%, ampicillin-sulbactam at 92.8%, piperacillin-tazobactam at 97.1%, cefotaxime at 97.7%, ceftazidime at 96.3%, ceftriaxone at 98.8%, imipenem at 97.5%, meropenem at 98.7%, ciprofloxacin at 98.8%, levofloxacin at 99.3%, chloramphenicol at 90.5%, trimethoprim-sulfamethoxazole at 50.6%, and tetracycline at 61.7% (NARST, 2020)⁽¹⁵⁾. In the present study, the authors found 49.28% of *H. influenzae* to be susceptible to ampicillin, 53.62% susceptible to trimethoprim-sulfamethoxazole, and 100% susceptible to cefotaxime, meropenem, and ciprofloxacin (Table 2). Therefore, the authors' *H. influenzae* isolates had lower susceptibility to ampicillin, but, slightly higher susceptibility to cefotaxime, meropenem, and ciprofloxacin when compared to NARST data.

There were potential limitations to the present study. First, data on *H. influenzae* isolates from a single study site was presented for an antibiogram profile. Second, the authors did not gather clinical information concerning vaccination against *H. influenzae* in patients. Finally, serotyping of *H. influenzae* isolates was not available because the isolates were not preserved in a -80°C freezer.

Conclusion

The present study found cefotaxime, ciprofloxacin, meropenem, and cefuroxime to be effective drugs in treating infections caused by *H. influenzae*. The present data should support ongoing studies evaluating current trends and help improve surveillance of drug resistance so that it is minimized. Furthermore, antibiogram profiles are necessary to avoid ineffective empirical drug treatments.

What is already known on this topic?

H. influenzae, which is one of the most common causes of community-acquired pneumonia, can cause various diseases, including meningitis and acute epiglottitis. According to the previous studies, drug resistance is a global problem. Therefore, a further study of *H. influenzae* prevalence and drug resistance should be conducted, particularly in Thai patients.

What this study adds?

H. influenzae found in 138 patients was isolated at Taksin Hospital between January 2016 and December 2021. The sites of *H. influenzae* included the sputum (71.01%), blood (11.59%), pus from eyes

(10.14%), and pus from urethra (2.90%). *H. influenzae* demonstrated susceptibility (100%) to cefotaxime, ciprofloxacin, and meropenem, but less susceptibility to cefuroxime (93.48%), ampicillin (49.28%), and trimethoprim-sulfamethoxazole (53.62%). This study demonstrates how antimicrobial susceptibility results can provide guidance for clinicians on treatment and prevention of *H. influenzae* diseases.

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Conflicts of interest

All authors declare no personal or professional conflicts of interest, and no financial support.

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