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Comparison of in vitro susceptibility of *Haemophilus influenzae* strains to various antimicrobial drugs

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ABSTRACT

Haemophilus species are the members of the normal flora of human upper respiratory tract. *Haemphilus influenzae* is the prominent organism in terms of morbidity and mortality. Haemophilus spp. cause serious infections that can progress to death. The aim of this study is to determine the frequency and antimicrobial susceptibility pattern of *H. influenzae* strains isolated from clinical samples between 2005 and 2010. *H. influenzae* strains from clinical samples were identified by BBL Crystal AutoReader or Vitek 2 Compact automated system. Antibiotic susceptibility tests were performed according to the standards of CLSI for *H. influenzae* strains. One hundred and fifty-eight *H. influenzae* strains isolated from clinical samples were included in the study. *H. influenzae* strains were isolated most commonly from samples sent by chest diseases clinics (25.3%). It was observed that *H. influenzae* was isolated most commonly from lower respiratory tract samples (sputum and tracheal aspirate). In the study period, 29.1% and 13.9% of the isolates were found to be resistant to trimethoprim sulfamethoxazole and ampicillin, respectively. Antibiotic resistance rate was 3.7% for ceftazidime and no resistance was detected for meropenem, imipenem and cefuroxime. We found a statistically significant difference in the resistance to ampicillin and trimethoprim sulfamethoxazole between 2005 and 2010 ($p<0.05$). But, there was no significant difference in the resistance to ceftazidime, chloramphenicol, aztreonam and rifampicin over the years ($p>0.05$). We suggest that trimethoprim-sulfamethoxazole and ampicillin are not appropriate options for the treatment of infections caused by *H. influenzae* in our hospital. Second generation cephalosporins and quinolones can be used for this purpose. We suggest that determination of the frequency of *H. influenzae* from clinical samples and its antibiotic susceptibility pattern may produce data for epidemiological studies and guide empirical antibiotic therapy.

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1. Introduction

Haemophilus influenzae is a non-spore forming gram negative rod that can only live in human being and has no other known natural host. The organism may be found in normal upper respiratory tract flora of children and adults. It may also colonize in nose and conjuctiva, and rarely genital tract. Human upper respiratory tract is colonized by non-typeable strains and less frequently by typeable strains including *H. influenzae* type b (Hib). While non-typeable strains can cause otitis media, acute exacerbation of chronic obstructive pulmonary disease, pneumonia, sinusitis, and sepsis, *H. influenzae* type b causes serious infections such as meningitis, epiglottitis and septic arthritis (Murphy, 2009). *H. influenzae* was one of the three major causes of bacterial meningitis in many countries until the implementation of widespread vaccination programs. Immunocompromised patients and children under three years of age who do not have adequate antibody levels have a high risk of infections caused by *H. influenzae* (Kilian, 2007). Hib is a significant pathogen for children and accounts for 95% of invasive *H. influenzae* infections. In particular, the organism is one of the most frequently isolated bacteria from meningitis at the age of 0-2 (Eşel et al., 2000). Unencapsulated strains cause infections mostly in adults with an underlying disease such as chron-

ic obstructive pulmonary disease or cystic fibrosis. The association of pneumonias primarily caused by *H. influenzae* with bacteremias has become increasingly important (Kilian, 2007). In addition to this increase, it is reported that there is also an increase in the ampicillin resistance. As well as the early diagnosis and management of these cases, more serious infections and potential complications can be prevented by appropriate empirical treatment. However, a great number of clinical isolates are resistant to β-lactam antibiotics, particularly ampicillin, chloramphenicol and tetracycline, basically due to dissemination of conjugative plasmids. The rate of ampicillin resistance of *H. influenzae* isolates ranges from 5% to 60% in different countries (Berkiten, 2004). Therefore, it is important to know local antibacterial resistance patterns to start appropriate emprical treatment.

In the study, we aimed to determine the distribution and antimicrobial susceptibility pattern of *H. influenzae* strains isolated from various clinical samples sent to our laboratory.

2. Materials and methods

H. influenzae strains isolated from various clinical samples sent to our laboratory from various clinics between 2005 and 2010 were included in the study. Samples admitted to our laboratory were inoculated on 5% sheep blood agar, eosin methylene blue (EMB) agar and chocolate agar. Following the incubation of culture plates at 37°C for 18 to 24 hours, organisms were evaluated in terms of colonial morphology and staining characteristics. Suspected *H. influenzae* colonies were identified by BBL Crystal autoReader (BD Diagnostic Systems USA) or Vitek 2 Compact automated system (bioMerieux SA France). A total of 158 *H. influenzae* strains were included in the study, duplicate isolates from one patient were removed. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion method according to the recommendations of Clinical and Laboratory Standards Institute (CLSI). *H. influenzae* ATCC 49247 and *H. influenzae* ATCC 49766 were used as the internal quality control strains. Statistical analysis was performed by using SPSS (Statistical Package for Social Scienses) 18.0 statistical program. Univariate chi-squared analysis was used to evaluate annual trends in antibiotic resistance.

3. Results

A total of 158 *H. influenzae* strains isolated from samples sent to Diagnostic Bacteriology Laboratory in the study period were included in the study. Gram stain of *Haemophilus influenzae* is shown in Figure 1. Growth of *Haemophilus influenzae* on chocolate agar is shown in Figure 2. Antibiotic susceptibility tests are shown in Figure 3 on the Haemophilus test medium (HTM).

H. influenzae strains were most frequently isolated from the samples from chest diseases clinic and secondly from internal medicine clinic (Table 1). *H. influenzae* was most frequently isolated from sputum samples. Clinical sample distribution (Table 2) and resistance pattern of isolated strains by years (Table 3) are given. Accordingly, there was a statistically significant difference between resistance to ampicillin and trimethoprim-sulfamethoxazole between 2005 and 2010 ($p<0.05$). But, there was no significant difference among resistance to ceftazidime, chloramphenicol, aztreonam and rifampicin by years ($p>0.05$).

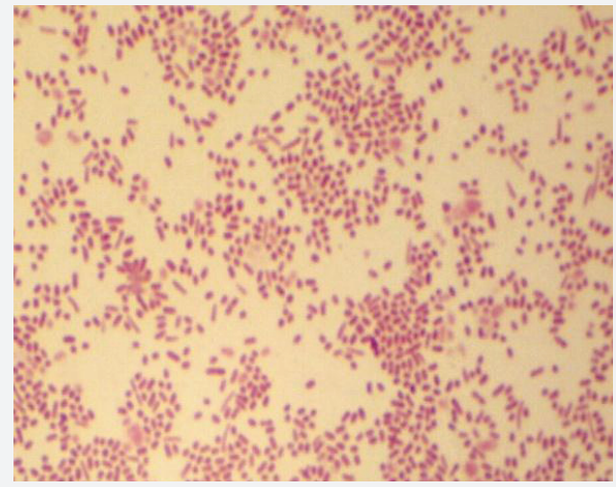

Fig. 1. Gram stain of *Haemophilus influenzae*

Fig. 2. Growth of *Haemophilus influenzae* on chocolate agar

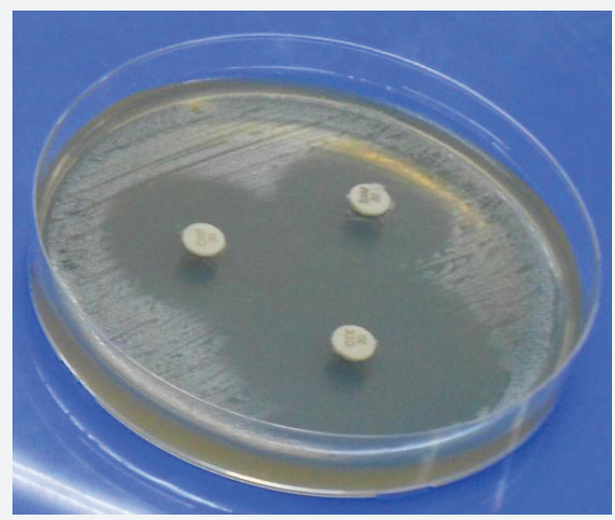

Fig. 3. The antimicrobial susceptibility of *Haemophilus influenzae* strain on Haemophilus test medium

4. Discussion

H. influenzae is an important pathogen that is commonly isolated from many parts of the world and can cause high morbidity and mortality (Tristram et al., 2007). It is important to know local resistance pattern in order to give an effective treatment. However, resistance rates vary among geographical regions. When studies from Turkey were examined; in the study by Şenol and Eriş (2000) the resistance rates of all isolates to ampicillin, erythromycin, ciprofloxacin, trimethoprim-sulfamethoxazole, tetracycline and chloramphenicol were found 46%, 48%, 16%, 36%, 31% and 13% respectively. In the study by İlki et al. (2010) between 2003 and 2006, resistance rates of *H. influenzae* isolates to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol and cefotaxime were found 3.3%, 25.5%, 2.2% and 0% respectively. In the study by Uncu et al. (2007) between 2005 and 2006, the authors found resistance rates of *H. influenzae* isolates to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, imipenem and cephalosporins 3.2%, 25%, 6.4%, 0%, 0%, 0% respectively. In a multicenter study, including 379 clinical isolates, by Şener et al. (2007) between 2004 and 2005, the rates of resistance to ampicillin, ofloxacin, tetracycline and trimethoprim-sulfamethoxazole were found 4.7%, 0%, 8.5% and 22.9%, respectively. While ampicillin resistance wasn't detected in some centers in the study, it was over 10% for many hospitals. In the study, including 861 clinical islates, by Altun and Gür (2008) from Ankara between 2002 and 2007, the rates of resistance to ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, meropenem and tetracycline were found 7.2%, 23%, 0%, 0% and 1.1%, respectively. According to the limited number of studies, ampicillin resistance in Turkey is between 0% and 52.5% (Şener et al., 2007). When we examine some of the studies abroad, ampicillin resistance varies between 5% and 60 % according to countries. Ampicillin and trimethoprim-sulfamethoxazole are excluded from the treatment due to changing or increasing resistance rates. Quinolone resistance is still rare (Kilian, 2007). In the study by Tristam et al. (2007) resistance rates of *H. influenzae* isolates to ampicillin, trimethoprim-sulfamethoxazole, cefuroxime, chloramphenicol and ciprofloxacin were found 17%, 17%, 0.7%, 1.9% and 0%, respectively. In the study by Ladhani et al. (2008) which investigated a period of twenty years in England, the authors found the resisitance rates to ampicillin, chloramphenicol and tetracyclin as 16.2%, 1.2% and 1.8%, respectively. In the study by Giufre et al. (2011) the resistance rates in H.influenzae to ampicillin, chloramphenicol, ciprofloxacin, cefotaxime and imipenem were found as 10.3%, 2.6%, 0%, 0% and 2.6%, respectively. In the study by Perez-Trallero et al. (2010) the authors found the resistance rates to ampicillin, cefuroxime, cefotaxime and ciprofloxacin as 13.3%, 0.1%, 0% and 0.2% respectively.

In the study by Cardines et al. (2010) the authors found the resistance rates to ampicillin, amoxicillin clavulanate, cefixime, cefotaxime, imipenem, ciprofloxacin, azithromycin and chloramphenicol in 79 H.influenzae strains isolated from patients with cystic fibrosis between 2004 and 2009 as 13.9%, 10.1%, 10.1%, 0%, 17.7%, 0%, 10.1% and 3.8%, respectively. In our study, carbapenem resistance was not detected.

In the study by Landhani et al. (2008) from England, the authors investigated a period of twenty years. While ampicillin resistance increased gradually from 1985 until the mid-

Table 1. Distribution of samples from which *H. influenzae* was isolated according to clinics

Clinics	Number of samples
Chest diseases	40
Internal medicine	36
Pediatrics	18
Intensive care unit	15
Cardiology	12
Emergency	9
Oncology	6
Chest surgery	5
Neurosurgery	4
Infectious diseases	3
Others	10

Table 2. Distribution of clinical samples from which *H. influenzae* was isolated according to years

Sample	2005	2006	2007	2008	2009	2010	Total
Sputum	12	8	33	24	20	11	108
Tracheal aspirate	0	4	6	5	7	3	25
Conjuctiva	1	2	5	6	1	1	16
Cerebrospinal Fluid	0	0	0	0	1	1	2
Others	1	1	3	0	1	1	7
Total	14	15	47	35	30	17	158

Table 3. Resistance pattern of *H. influenzae* according to years

Year/Antibiotic (n,%)	AMP	SXT	CAZ	C	MEM	IMP	CXM	AZT	TE	CIP	RA
2005 14, (8.8)	1 (7.1)	4 (28.5)	1 (7.1)	0	0	0	0	1 (7.1)	0	0	1 (7.1)
2006 15, (9.4)	1 (6.6)	1 (6.6)	0	0	0	0	0	0	0	0	0
2007 47, (29.7)	5 (10.6)	10 (21.2)	1 (2.1)	0	0	0	0	2 (4.2)	4 (8.5)	0	1 (2.1)
2008 35, (22.1)	6 (17.1)	12 (34.2)	3 (8.5)	2 (5.7)	0	0	0	0	0	1 (2.8)	0
2009 30, (18.9)	9 (30)	12 (40)	0	1 (3.3)	0	0	0	0	0	0	0
2010 17, (10.7)	0	7 (41.1)	1 (5.8)	0	0	0	0	0	0	0	0
Total 158	22 (13.9)	46 (29.1)	6 (3.7)	3 (1.8)	0	0	0	3 (1.8)	4 (2.5)	1 (0.6)	2 (1.2)
P	0.000	0.000	0.228	0.317				0.366		0.096	

AMP: Ampicillin; SXT: Trimethoprim sulfamethoxazole; CAZ: Ceftazidime; C: Chloramphenicol; MEM: Meropenem; IMP: Imipenem; CXM: Cefuroxim; AZT: Aztreonam; TE: Tetracycline; CIP: Ciprofloxacin; RA: Rifampicin; NOTEb: Cefuroxime was not performed for the strains resistant to ceftazidime

1990s, it declined to the lowest ratio of 11.6% in 2004. While trimethoprim-sulfamethoxazole resistance was 10% in 1985, it decreased until 1990 then increased gradually to 11.9% in 2004. In the study of 578 isolates by Jansen et al. (2006) amoxicillin resistance was reported 19.8%, 23.3% and 16.4% for 1997/98, 2002/03 and 2004/05, respectively. In the study by Doern et al. (1997) from USA that included 1537 clinical isolates between 1994 and 1995, ampicillin and trimethoprim-sulfamethoxazole resistance were reported 38.9% and 9%, respectively. In the study by Harrison et al. (2009) from USA that included 143 clinical isolates between 2005 and 2007, amoxicillin and trimethoprim-sulfamethoxazole resistances were reported 32% and 26.6%, respectively. In the study by Cobos et al. (2008) from Spain that included 197 clinical isolates between 1997 and 2007, there was a gradual decline in the amoxicillin resistance from 38.6% to 30.2% and trimethoprim-sulfamethoxazole resistance from 50% to 34.9%. In our study, *H. influenzae* was most commonly isolated from lower respiratory tract samples (sputum and tracheal aspirate). Resistance of clinical isolates to ampicillin (13.9%) and trimethoprim-sulfamethoxazole (29.1%) was found higher compared to other antibiotics. Results were consistent with literature data. In our study, while ampicillin resistance increased between 2005 and 2009, the resistance wasn't seen in the strains isolated in 2010. The change in these years was statistically different ($p<0.05$). The absence of ampicillin resistance in 2010 may be due to the antibiotic policies in our hospital, but the small number of isolates should be considered. Trimethoprim-sulfamethoxazole resistance decreased in 2006, whereas it increased in the other years. Increase and change in the trimethoprim-sulfamethoxazole resistance in years were found statistically significant ($p<0.05$). Because cefuroxime wasn't performed for the isolates which was resistant to ceftazidime, cefuroxime susceptibility couldn't determined in these strains. There were no imipenem and meropenem resistance. For tetracycline, chloramphenicol, aztreonam and rifampicin, very low level of resistance was detected in some years.

Minimal resistance to quinolones has been reported in the studies from Turkey and abroad. In the studies from Turkey, it is seen that very different results, sometimes even different from expected, were obtained. This situation may be due to geographical differences, patient groups, methodologies and knowledge and experience of the authors.

Considering our resistance rates, in the empirical treatment of the patients with lower respiratory tract infection, we suggest that trimethoprim-sulfamethoxazole and ampicillin are not viable options. Cefuroxime, ceftazidime, and ciprofloxacin are among the options that can be used firstly for this purpose. We suggest that it is important to determine the frequency of isolation and antibiotic resistance pattern of *H. influenzae* periodically in terms of providing data to epidemiological studies and guiding empirical antibiotic treatment.

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